

These supplementary materials belong to:

A VersaTile approach to reprogram and broaden the specificity of R2-tailocins towards *E. coli* and *K. pneumoniae* serotypes

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Supplementary Figures and Tables

Supplementary Table S1. Amino acid sequences of chimeric receptor-binding proteins (RBPs).

Amino acid protein sequence in FASTA format
<p>>Tail_fiber_R2prf15</p> <p>MTTNTPKYGGLLTDIGAAALATASAAGKKWQPTHMLIGDAGGAPGDTPDPLPSAAQKSLINQRHRAQLNRLFVSDKNANTLVAEVLVPVEVG GFWIREIGLQDADGKFVAVSNCPSPSYKAAMESGSARTQTIRVNIALSGLENVQLLDNGI IYATQDWVKEKVAADF KGRKILAGNGLLGGGD LSADRSIGLAPSGVTTAGSYRSVTVNANGVVTTQGSNPTTLAGYAIGDAYTKADTDGKLAQKANKATTLAGYGITDALRVDGNAVSSSRLAAPR SLAASGDASWSVTFDGSANVSAPLSLSATGVAAGSYPKVTVDTKGRVTAGMALAATDIPGLDASKLVSGVLAEQRLPVFARGLATAVSNSSD PNTATVPLMLTNHANGPVAGRYFYIQSMFYPDQNGNASQIATSYNATSEMYVRVSYAANPSIREWLPWQRCDIGGSFTKEADGELPGGVNLD SMVTSGWWSQSFTAQAASGANYPIVRAGLLHVYAASSNFIYQTYQAYDGESFYFRCHSNTWFPWRRMWHGGDFNPSDYLLKSGFYWNALPG KPATFPSPSAHNHDVGQLTSGILPLARGGVGSNTAAGARSTIGAGVPATASLGASGWWRDNDTGLIRQWGQVTCPADADASITFPIPFPTLCL GGYANQTSAFHPGTDASTGFRGATTTTAVIRNGYFAQAVLSWEAFGR</p> <p>>Chaperone_R2prf16</p> <p>MGAGHLPRRCRCFDYVPDSFPYAMPRRICESDECFPSGNGCQYRFPWSDYHYRGDSQWLLCSGGSFMGGIWTMKGEYFSPSQVAFYPASLR EVYEYAGCWPVDGEWVS AELHEQLMNEQAAGRAISSDVNGNPVAIERPPLSRQQRSTHERRWRDSQLLATDGLVVRHRDQLETGKETTLFPV QYHELMSYRASLRDWPEEPLFPDSSGRPSVPDWLRRYVTF</p>
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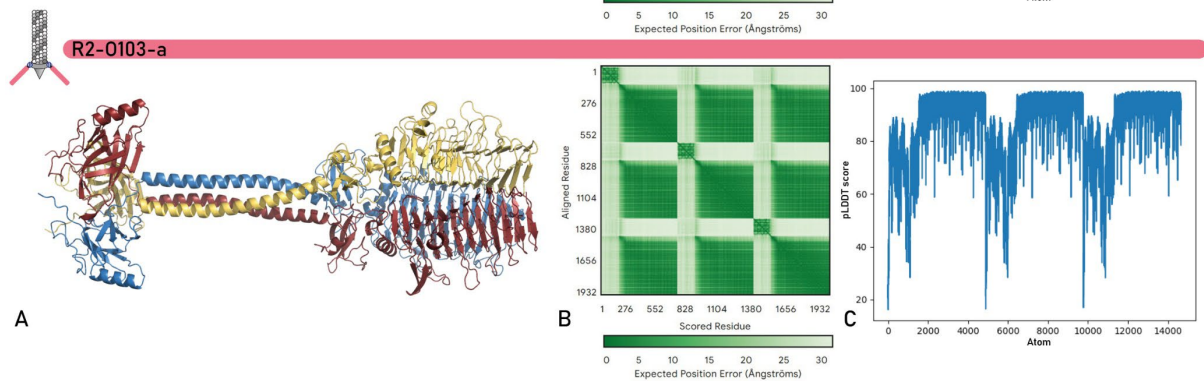
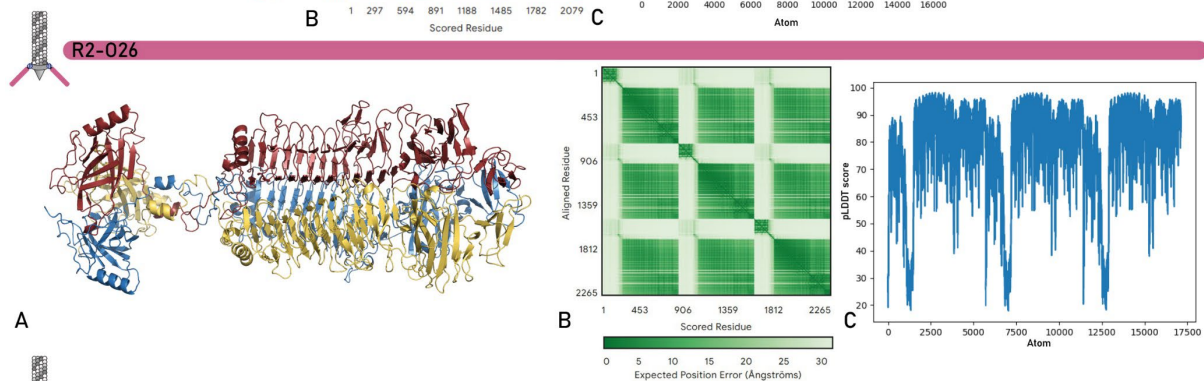
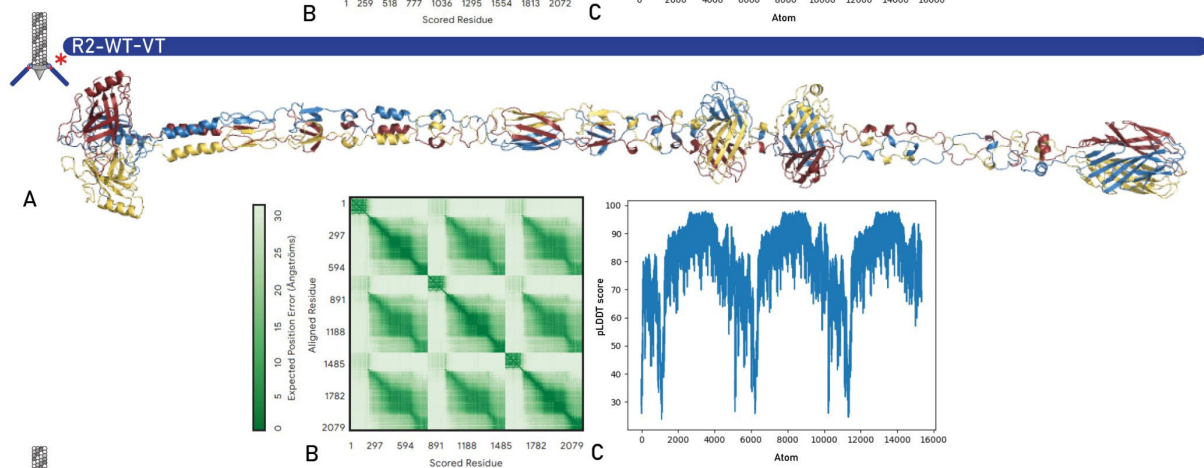
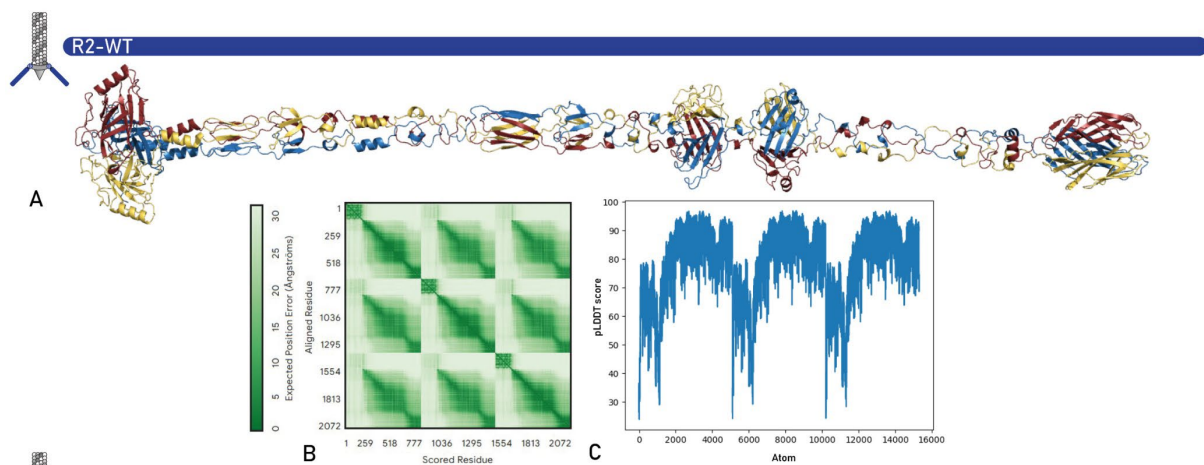
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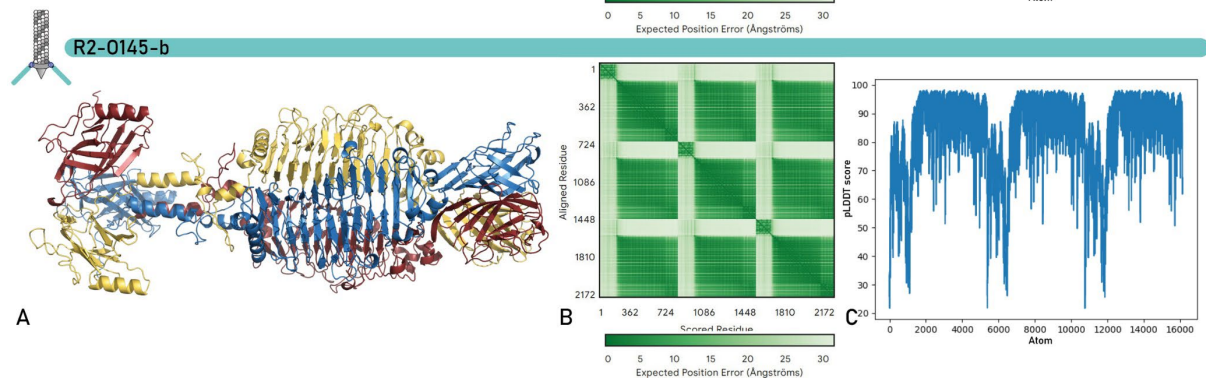
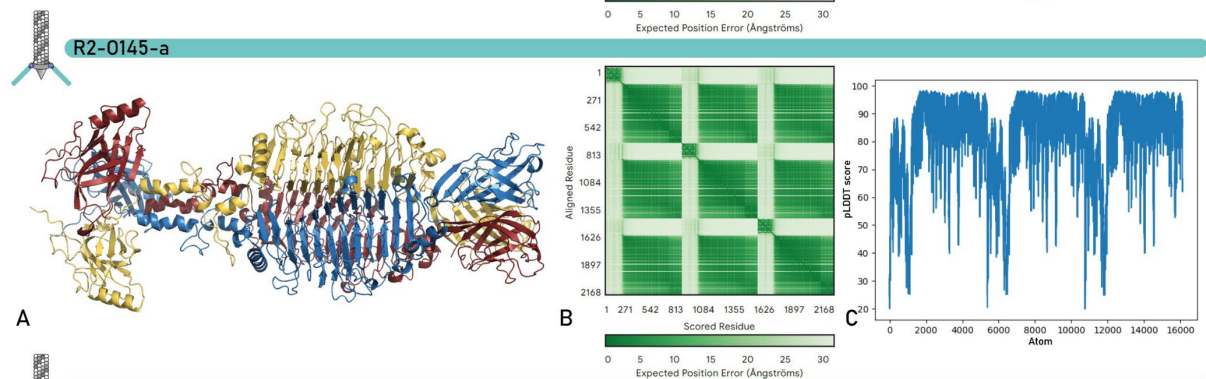
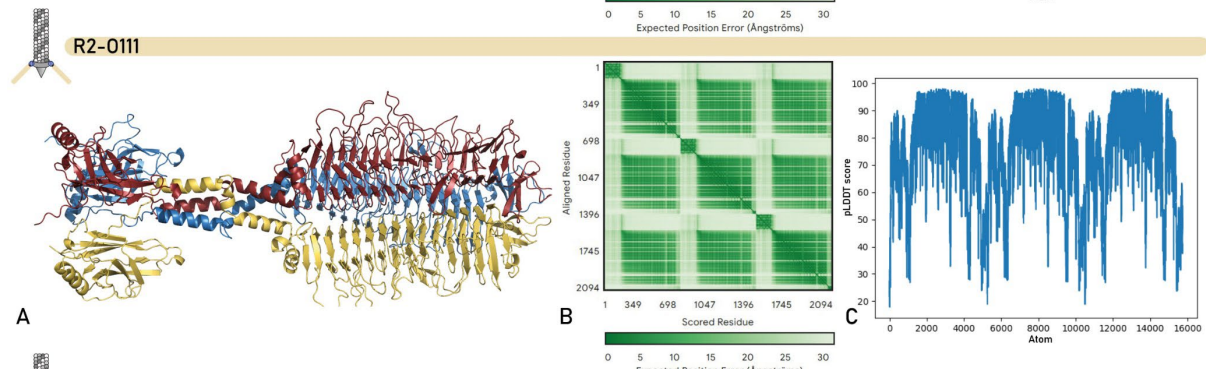
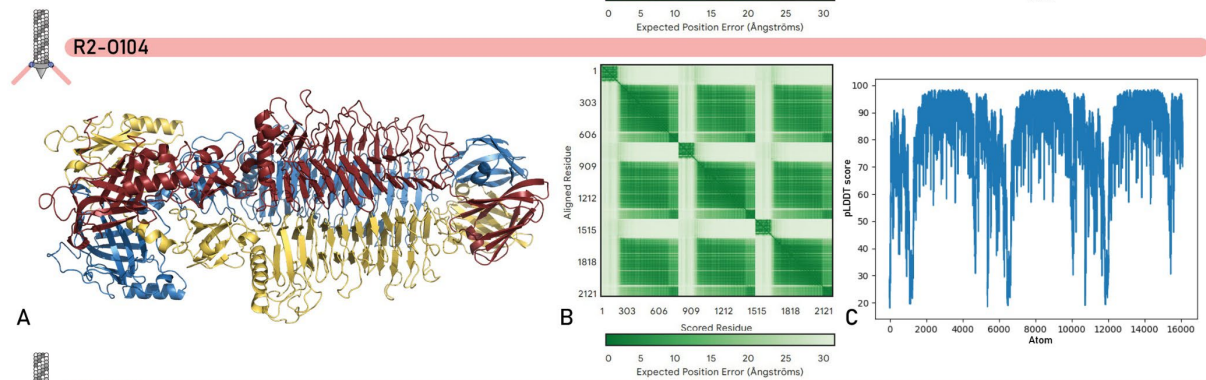
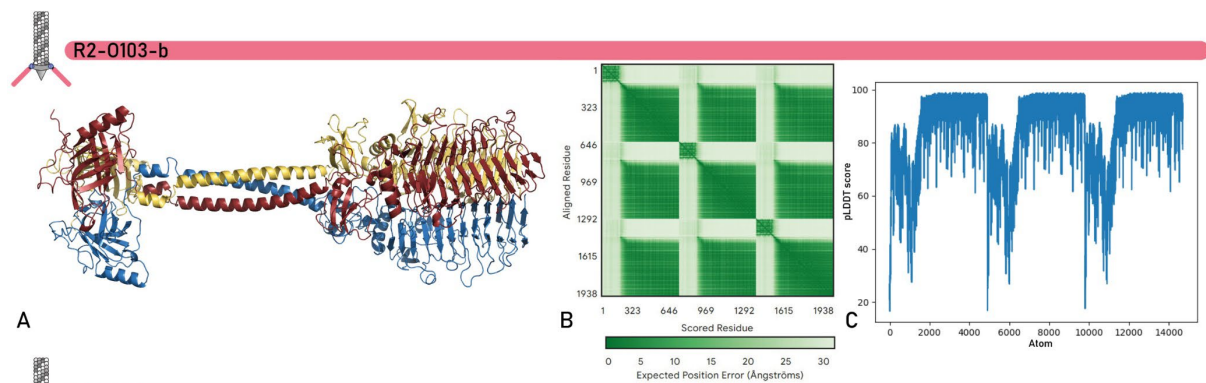
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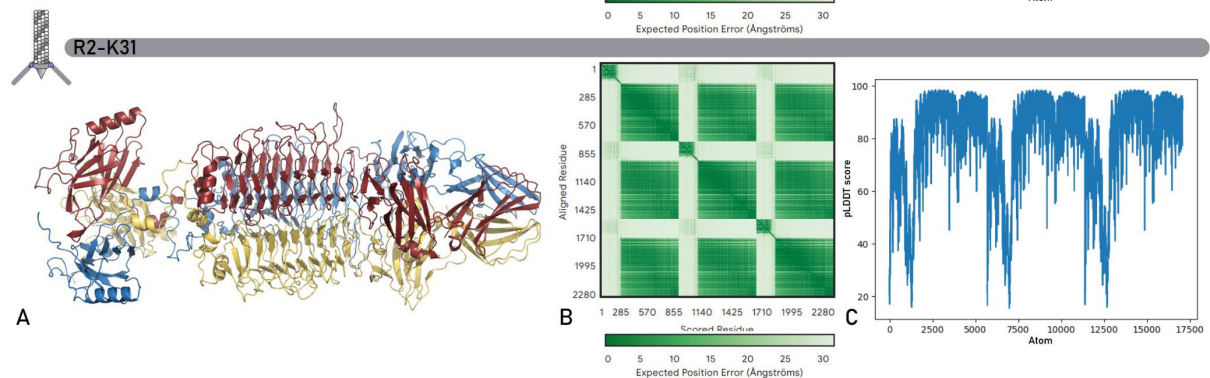
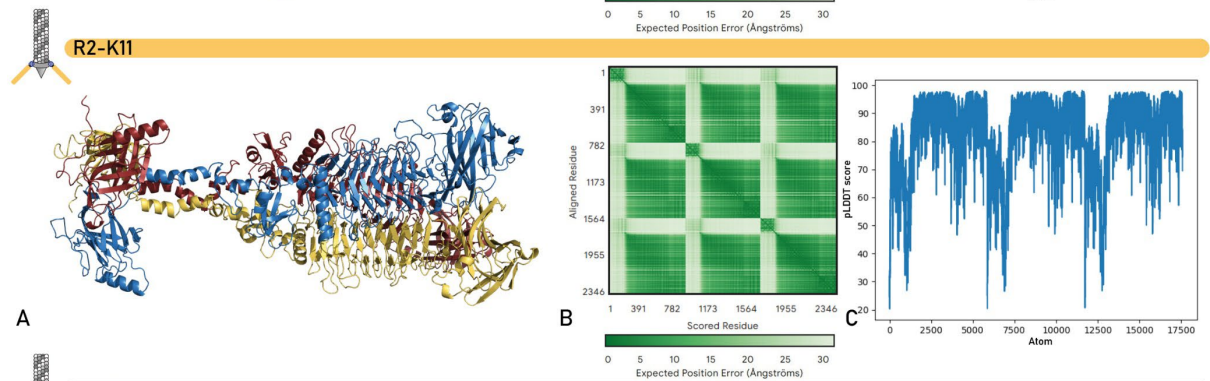
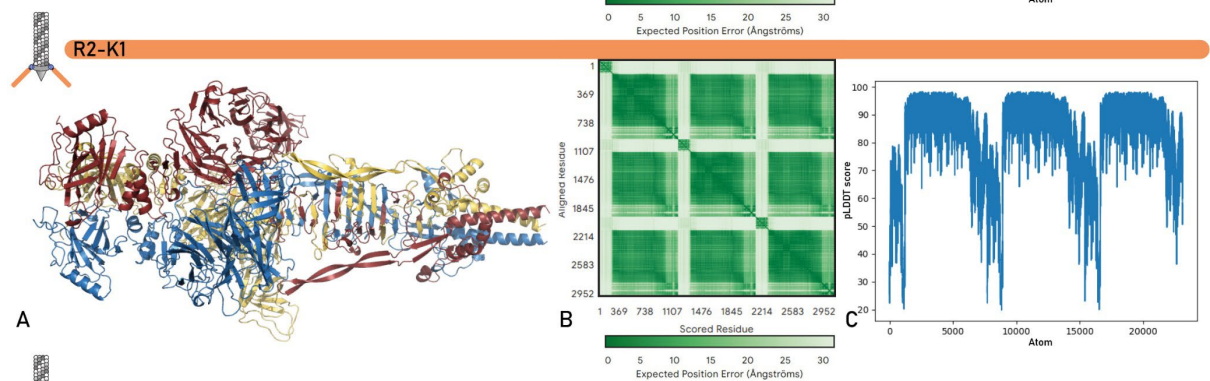
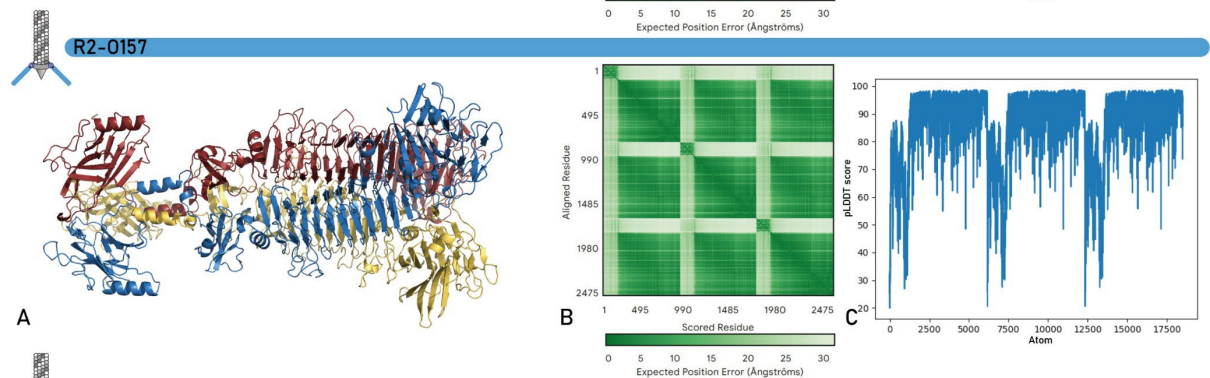
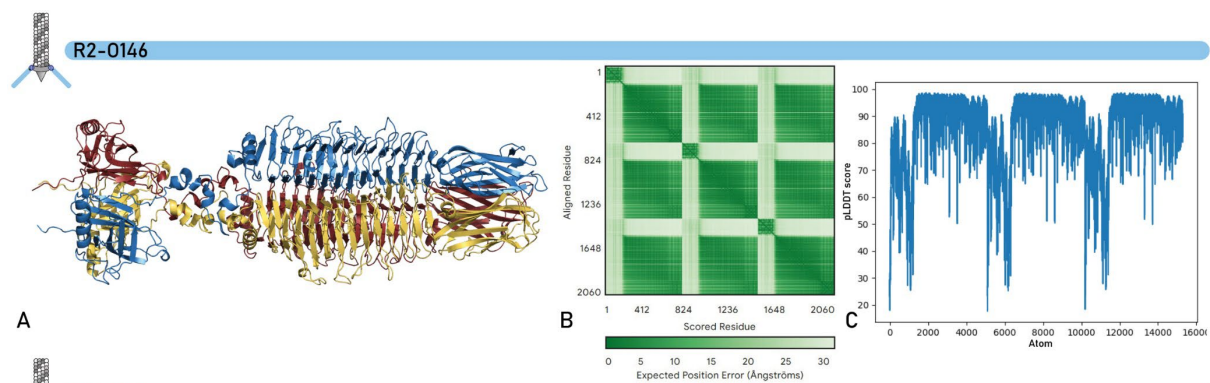
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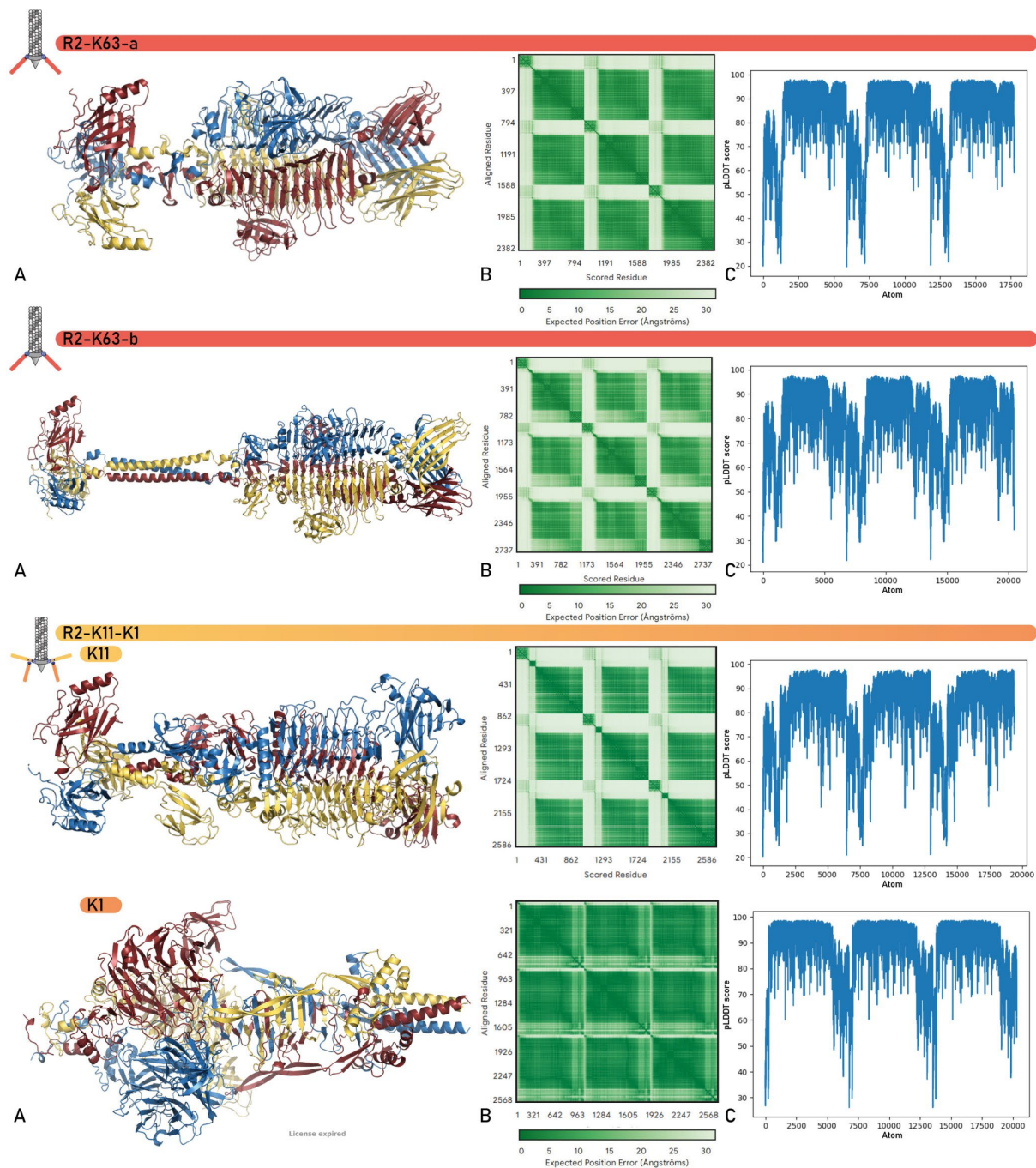
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PASSYTAGQLIFTNRSDTVFKLDGVRVSRADFVDVTIAWSPTPISAGSVVNTTASITRVSSHVVGTSGLKTDGTLGGAVSSSYFNRGANTL VVQLAALTAATPSITQVTVRLFLN
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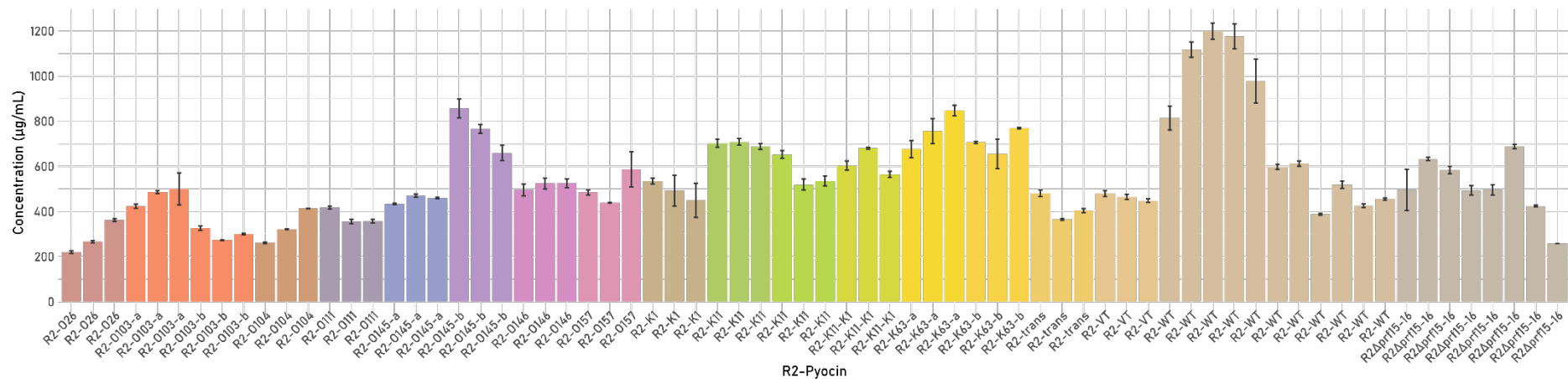




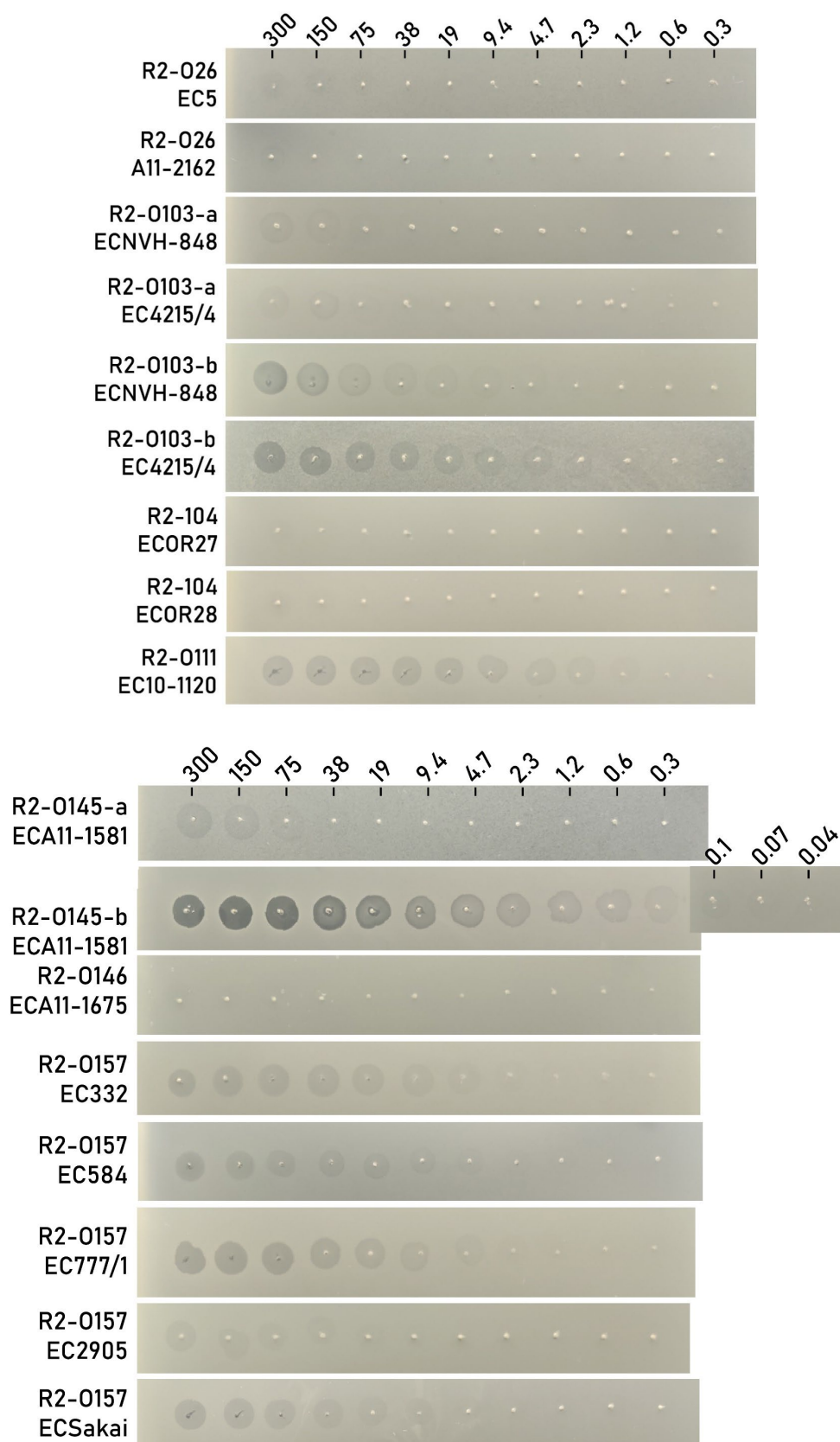


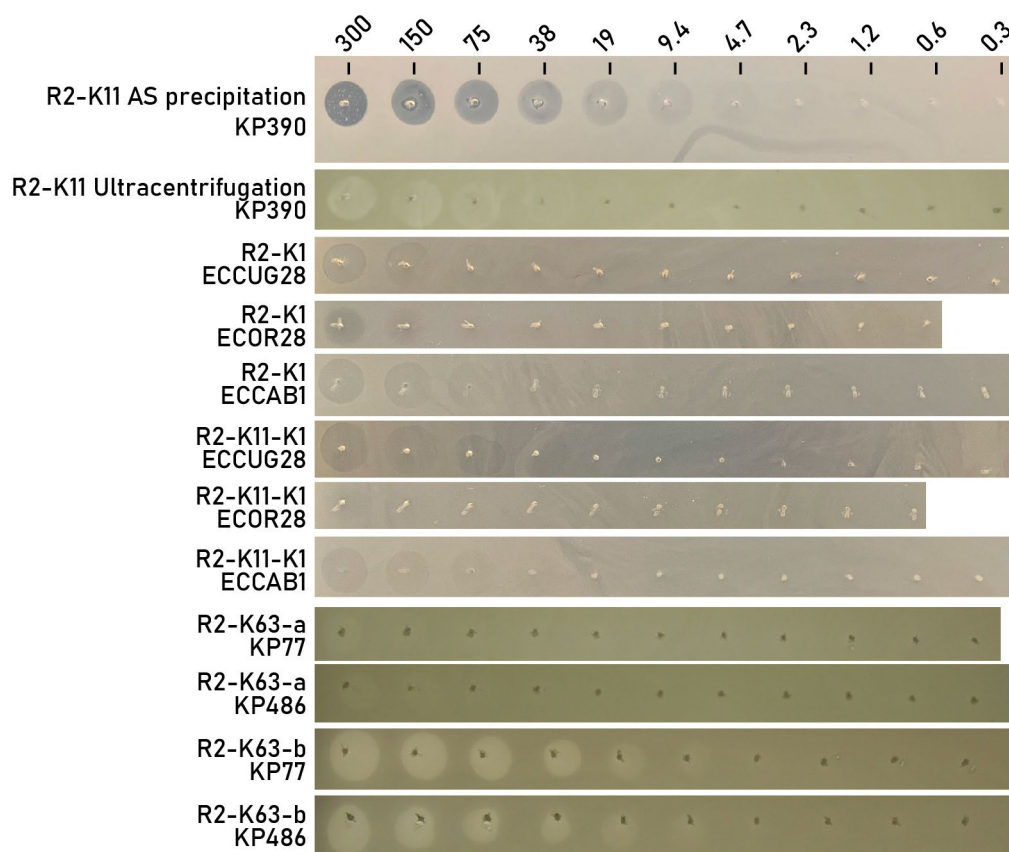


Supplementary Figure S1. AlphaFold3 predicted receptor-binding protein (RBP) structures. Each chimeric RBP is shown according to their target O-antigen/capsule serotype. **(A)** AlphaFold3 structure of trimeric RBP with each trimer indicated in a different color. **(B)** AlphaFold3 expected error per amino acid residue of the RBP. **(C)** Predicted Local Distance Difference Test (pLDDT) scores per atom within the RBP.

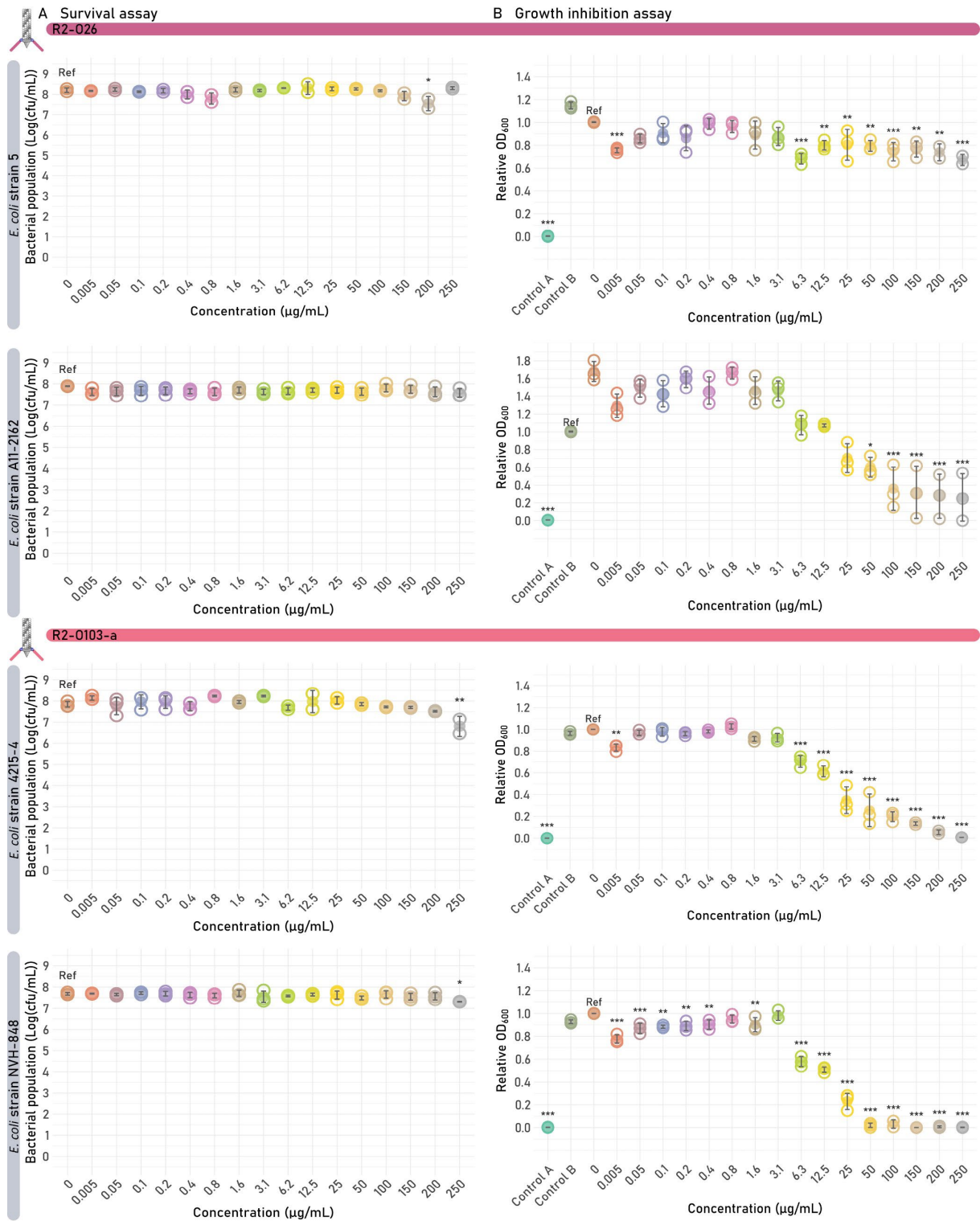


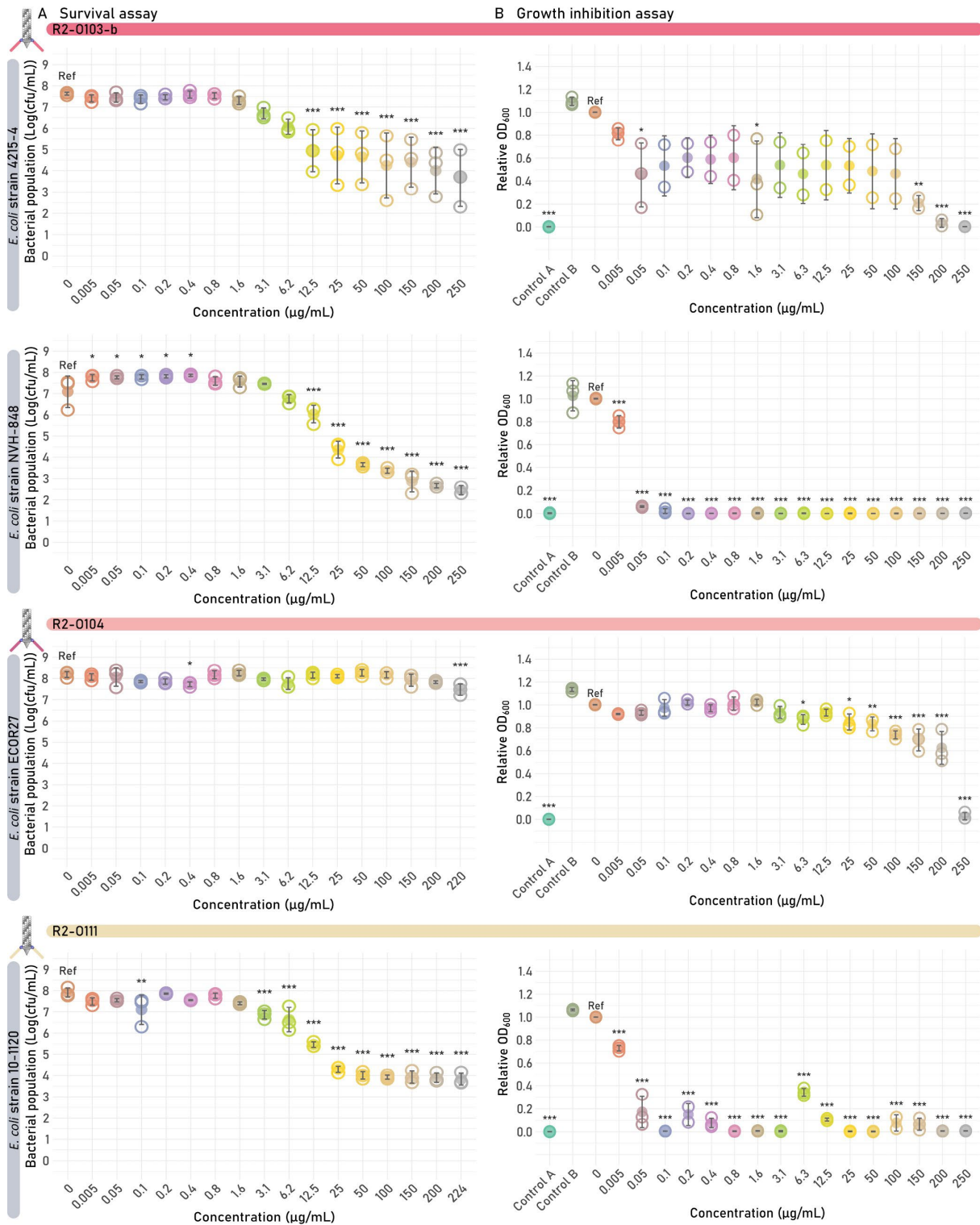
Supplementary Figure S2. An overview of the protein concentrations for each biological replicate of produced engineered R2 tailocin. Protein concentrations of the expressed (engineered) R2 tailocins were measured with the Micro BCA™ Protein Assay Kit (Thermo Fisher Scientific) after purification with ammonium sulfate (AS) precipitation or high-speed centrifugation. The error bars indicate the standard deviation of technical triplicate measurements.

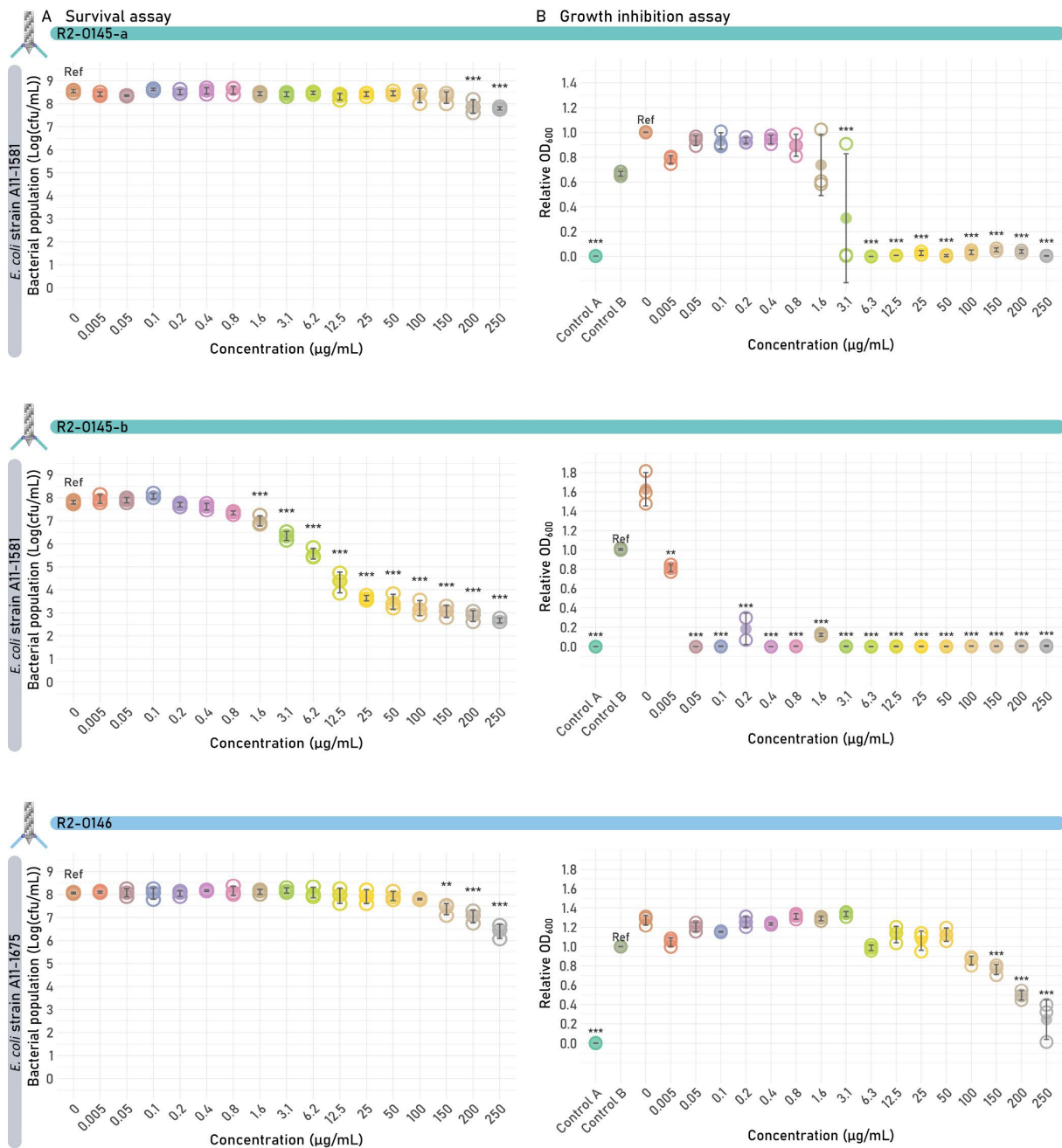


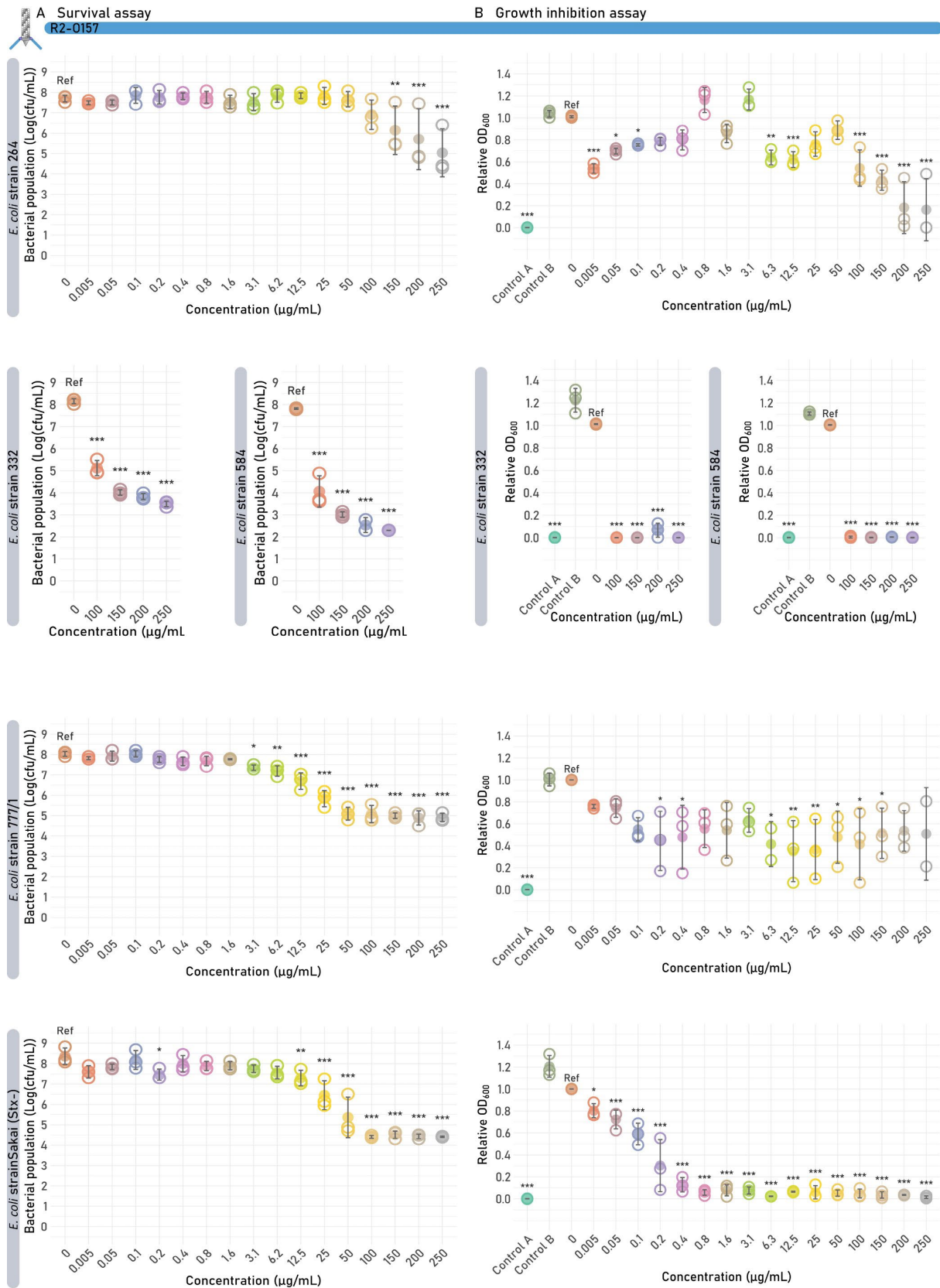


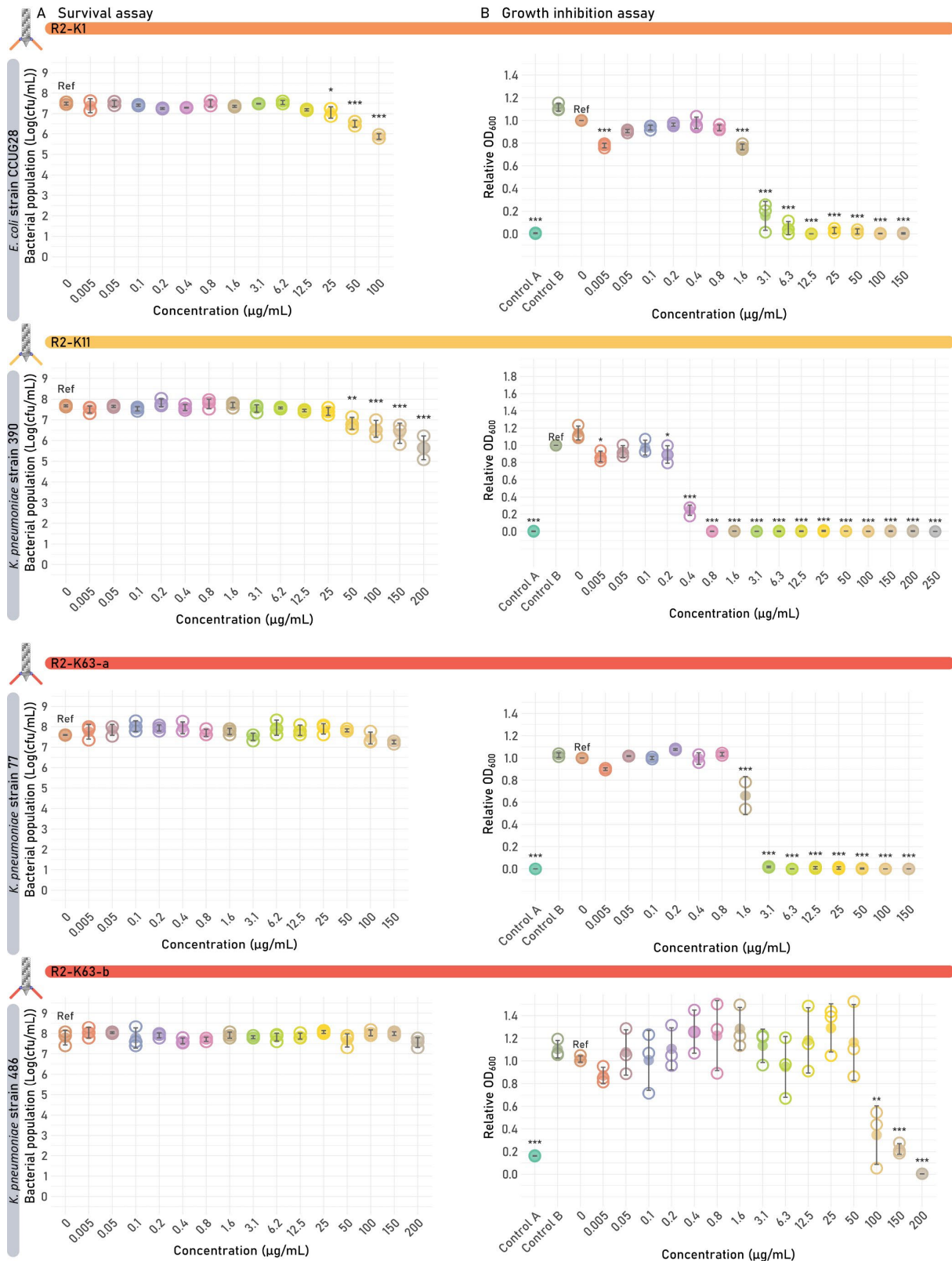
Supplementary Figure S3. Results of the two-fold serial dilution spotting assays of engineered R2 tailocins. The name of the engineered R2 tailocin construct and strain used to perform the spotting are stated on the left side of the figures. Volumes of 5 μL were spotted in increasing dilutions, from 300 to 0.04 $\mu\text{g}/\mu\text{L}$.

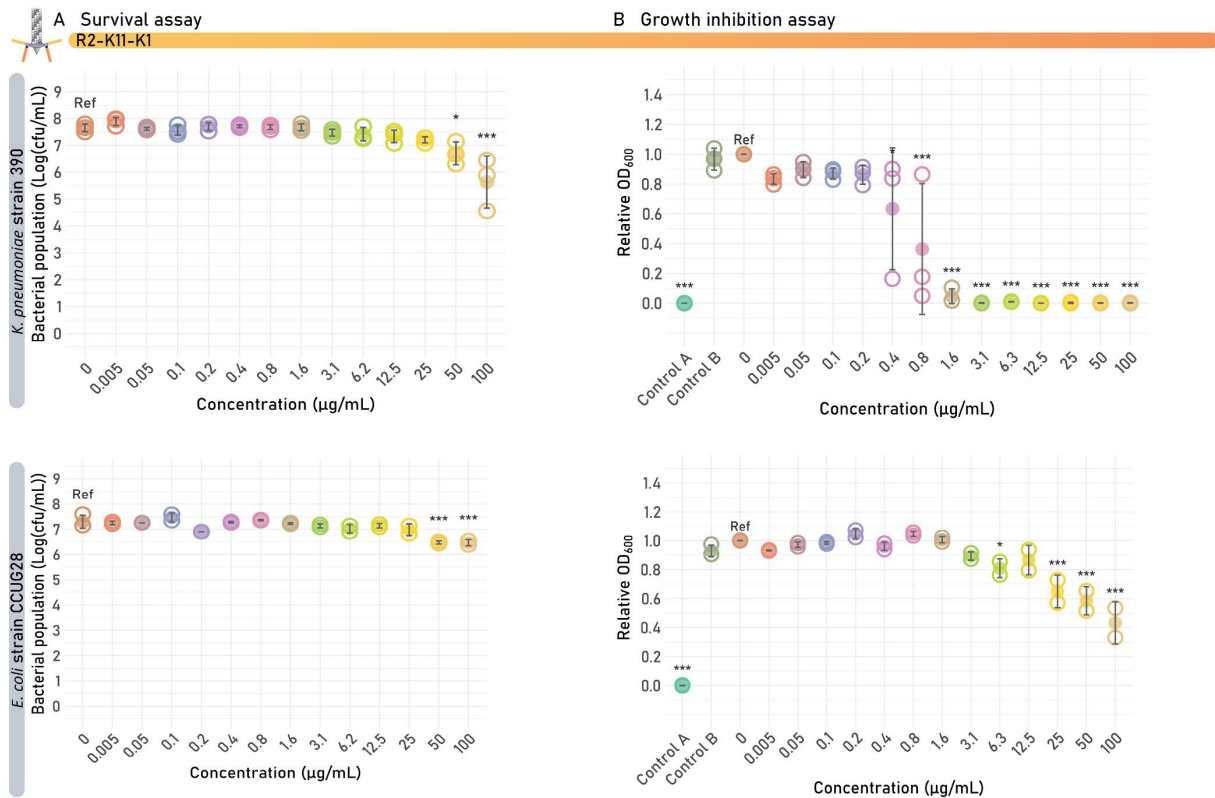






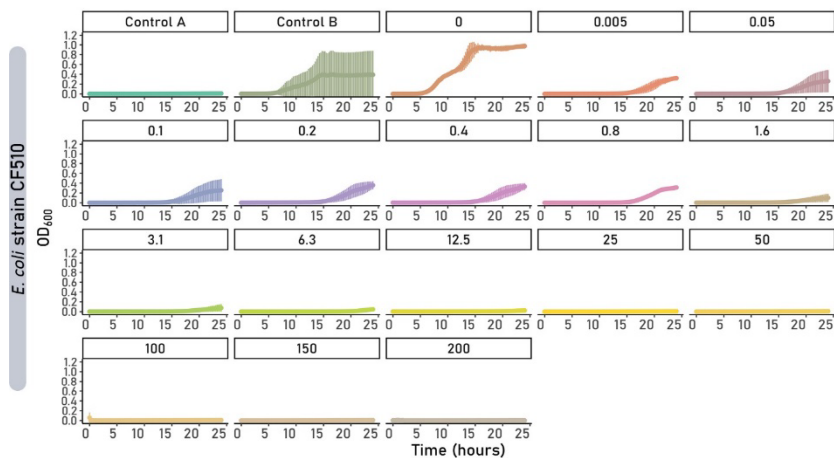




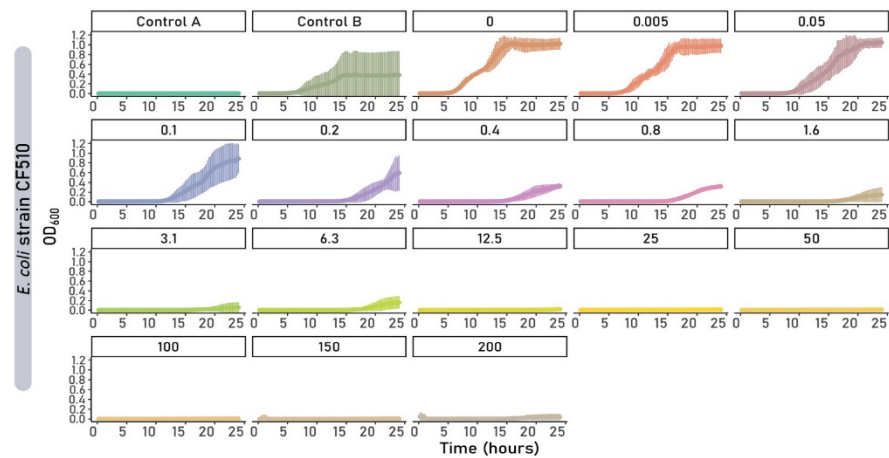


Supplementary Figure S4. Survival and growth inhibition assays of the different engineered R2 tailocins. Each R2 tailocin was tested on their susceptible target strains. Significant differences are shown by asterisks (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$). Reference (ref) indicates the value that was used as a reference for statistical comparison (untreated sample or control B). **(A)** Results of the survival assay per R2 tailocin construct and tested target strain, showing the bacterial colony count in function of the concentration of the added R2 tailocin. The value of each biological replicate is displayed using open circles and the mean values are shown as filled circles. **(B)** Growth inhibition assay results at 8 h are shown per R2 tailocin construct and tested target strain. The relative OD₆₀₀ of each biological replicate is displayed using open circles, and the mean relative OD₆₀₀ is shown as full circles. Two additional controls were performed, one containing the R2 tailocin but without the bacterial strain (Control A) and one containing a receptor-binding protein (RBP)-lacking mutant R2 tailocin particle (R2 $\Delta prf15$) (Control B). Both controls were added at the highest available R2 tailocin concentration (220-250 μg/mL).

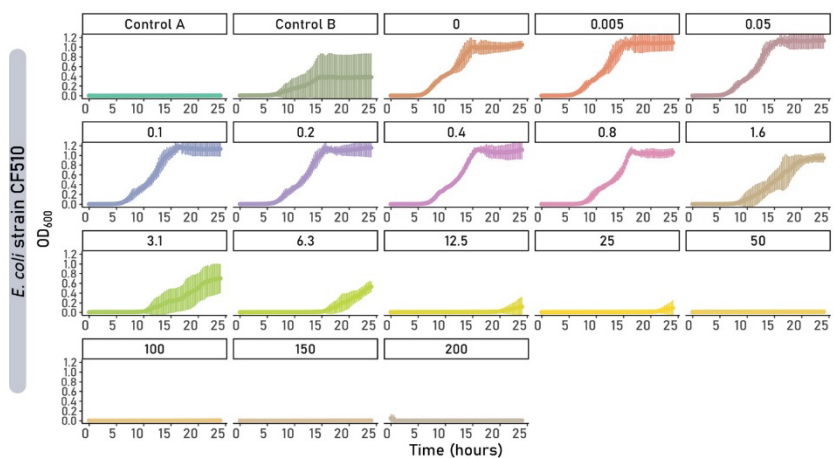
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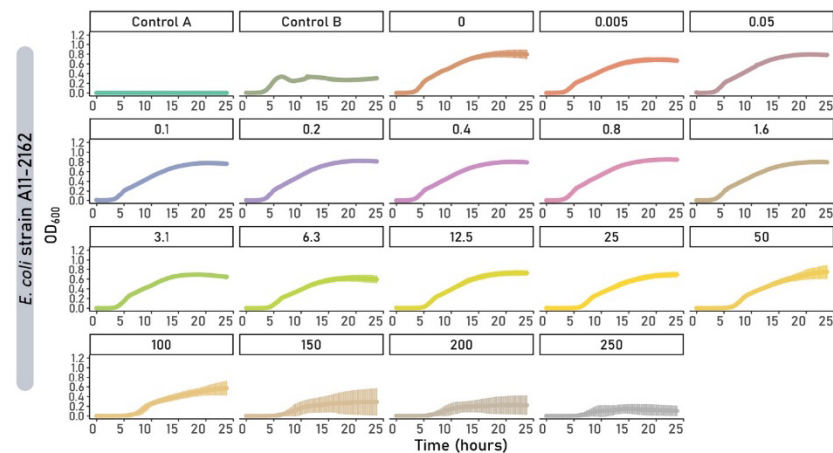
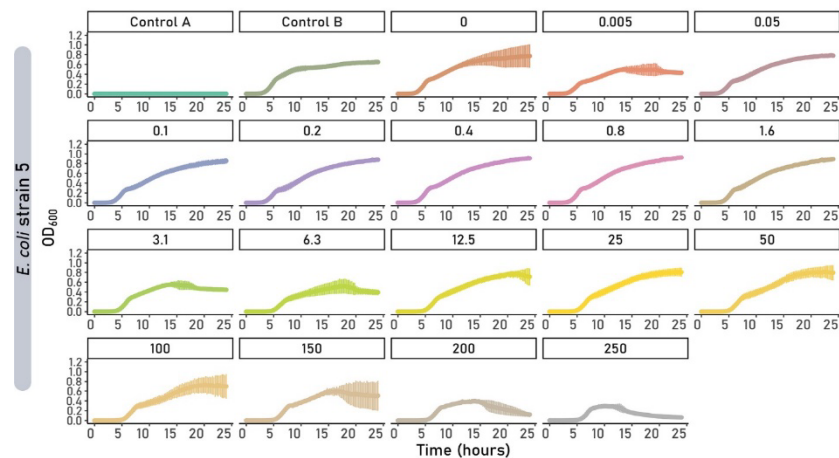
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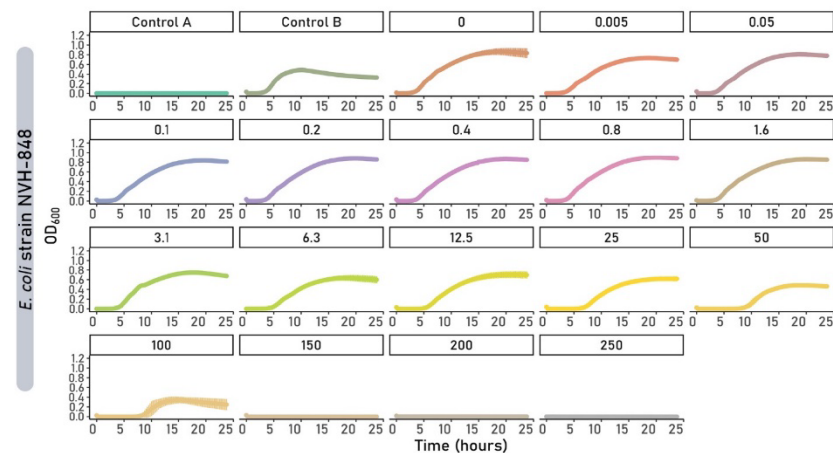
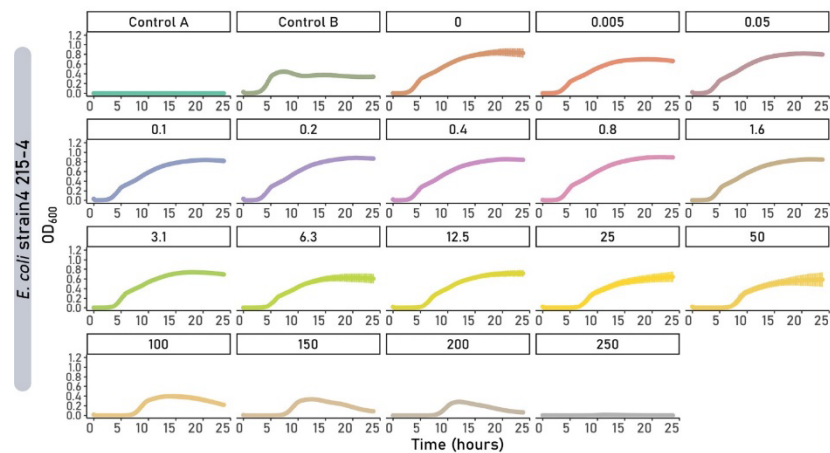
R2-WT-VT



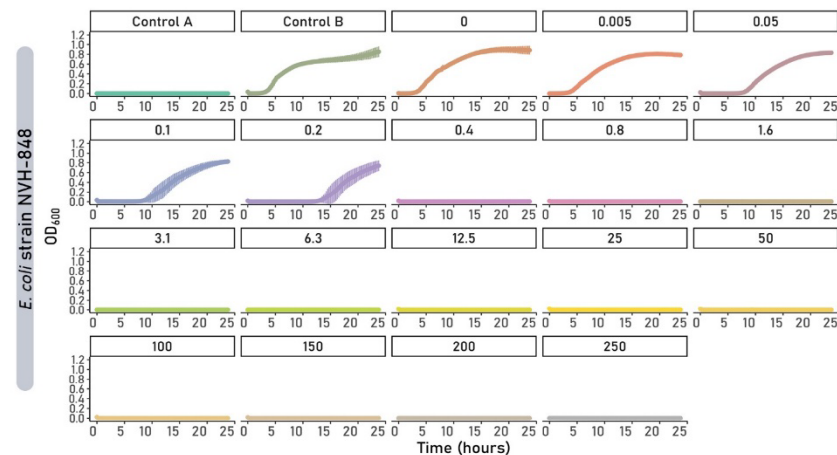
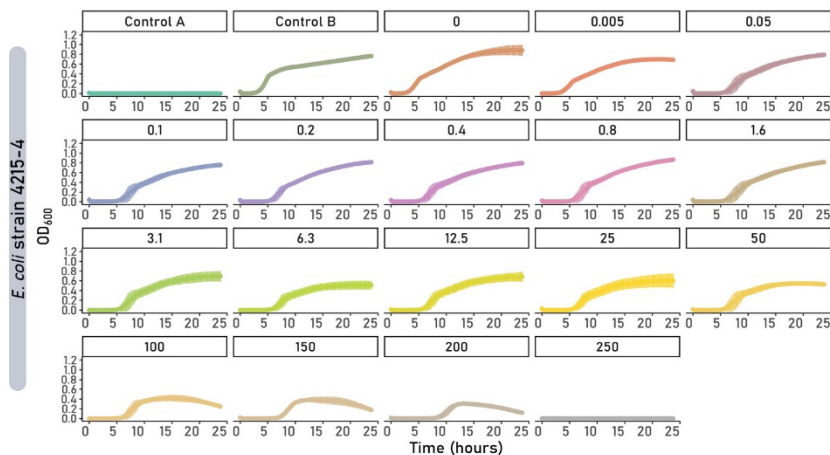
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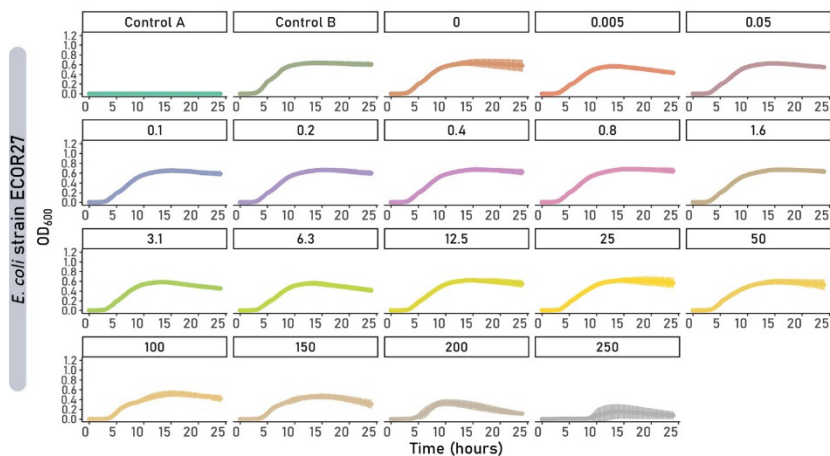
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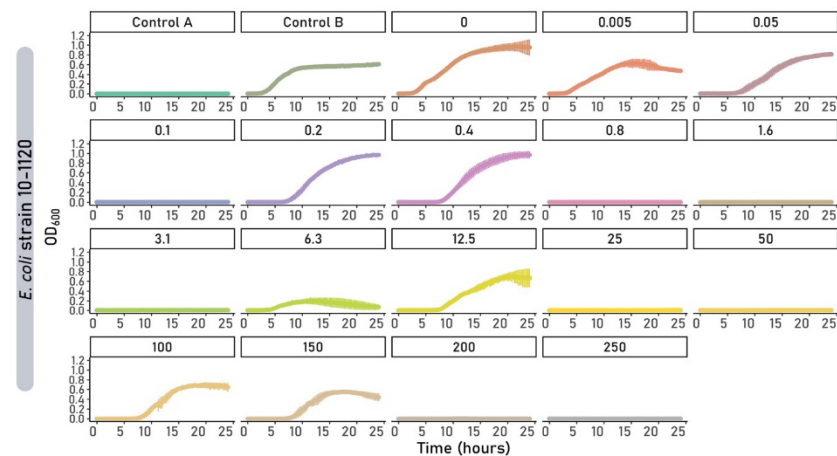
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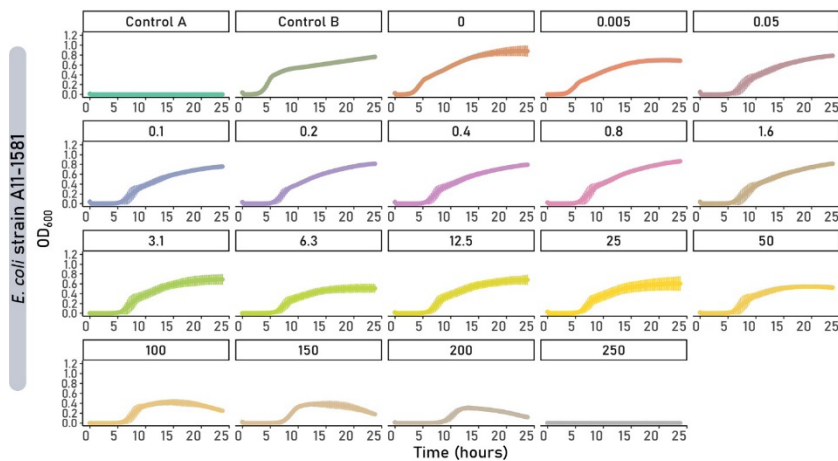
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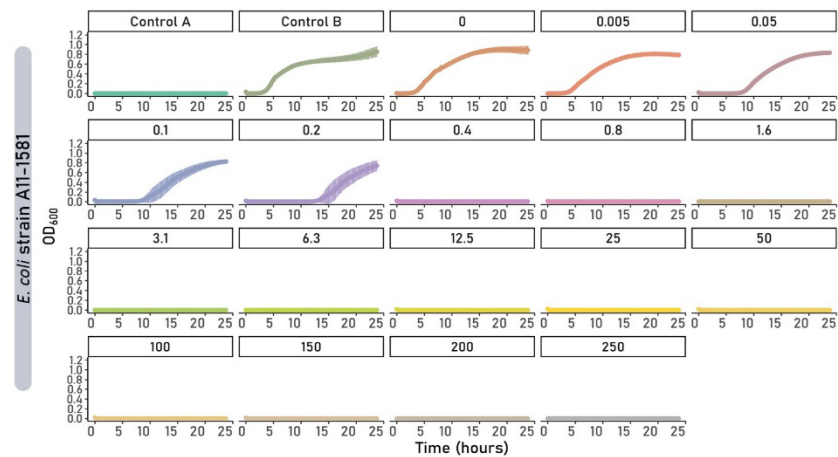
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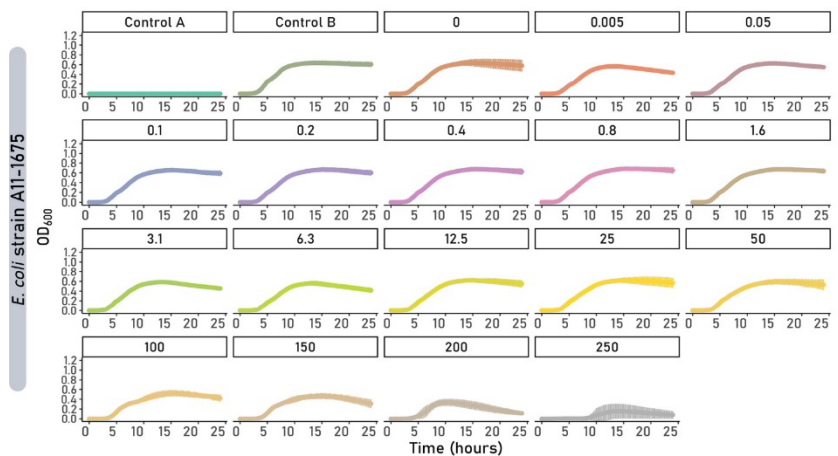
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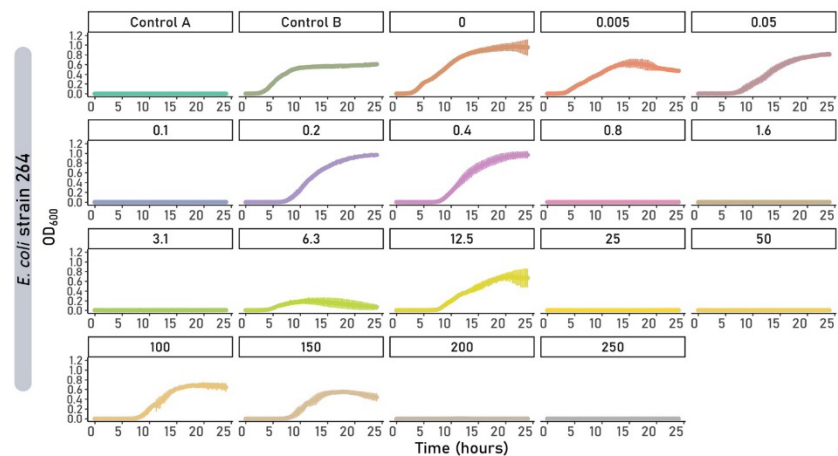
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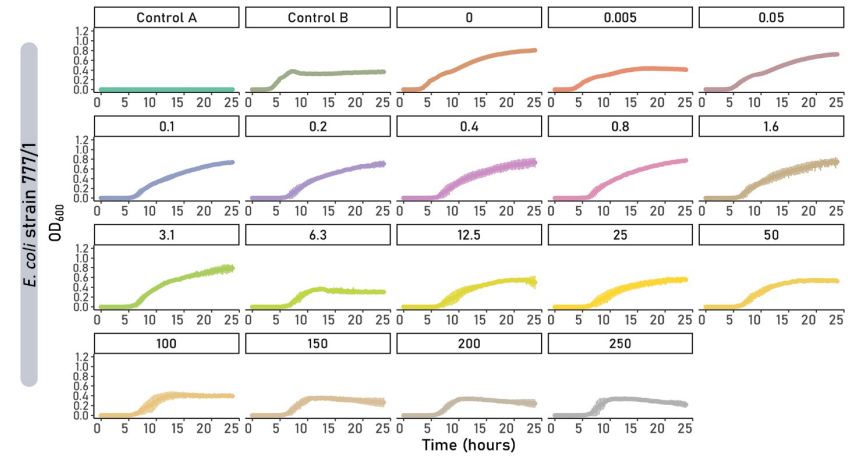
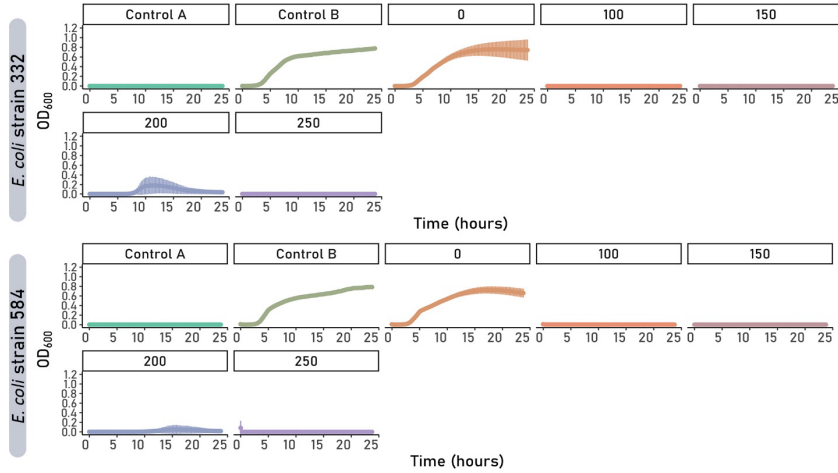
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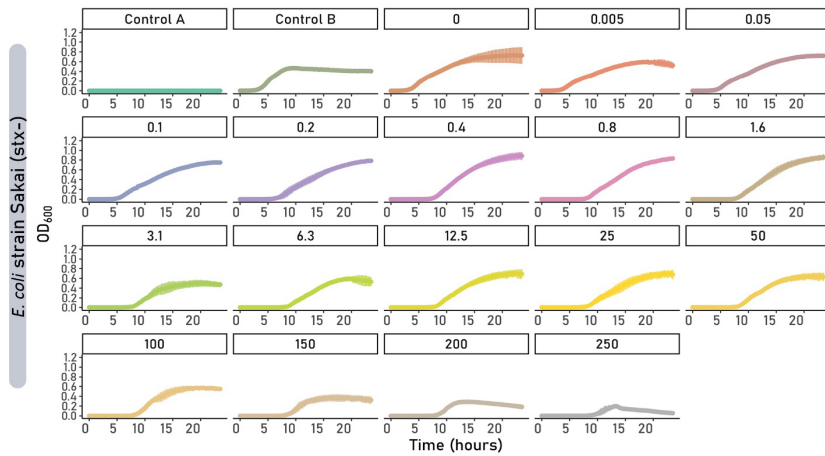
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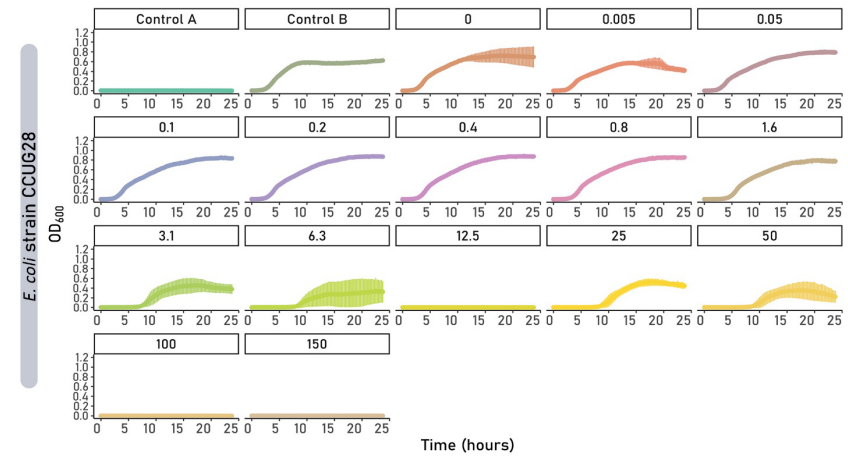
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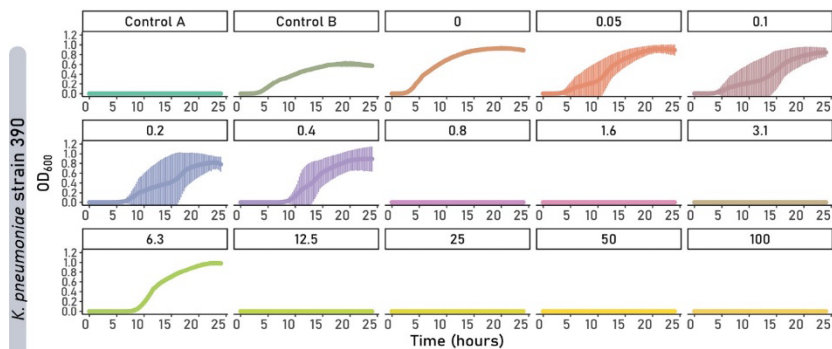
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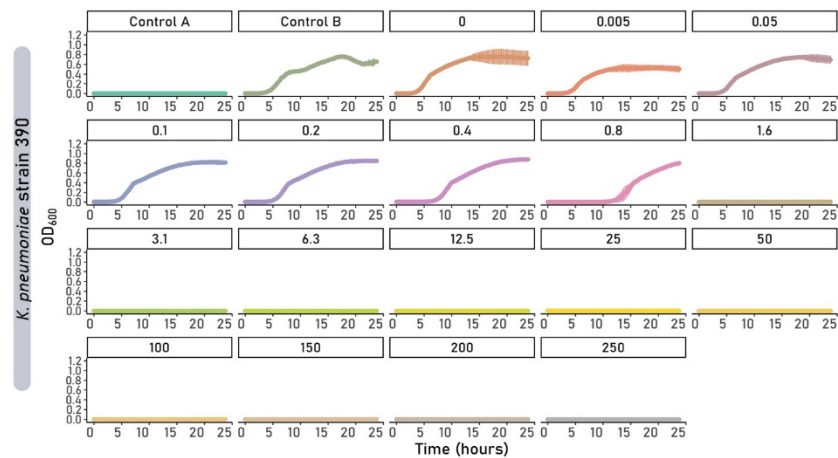
R2-K1



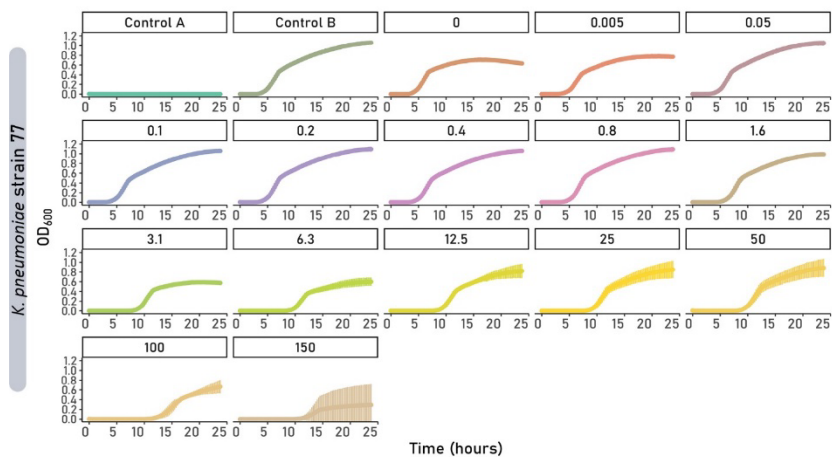
R2-K11 AS precipitation



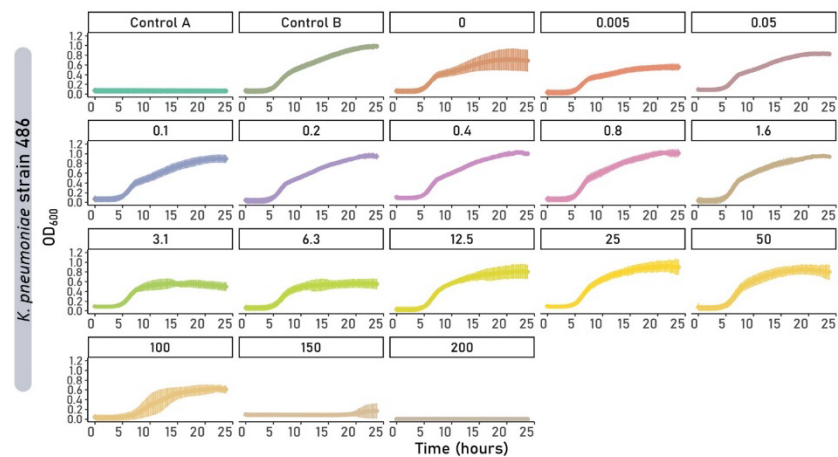
R2-K11 High-speed centrifugation

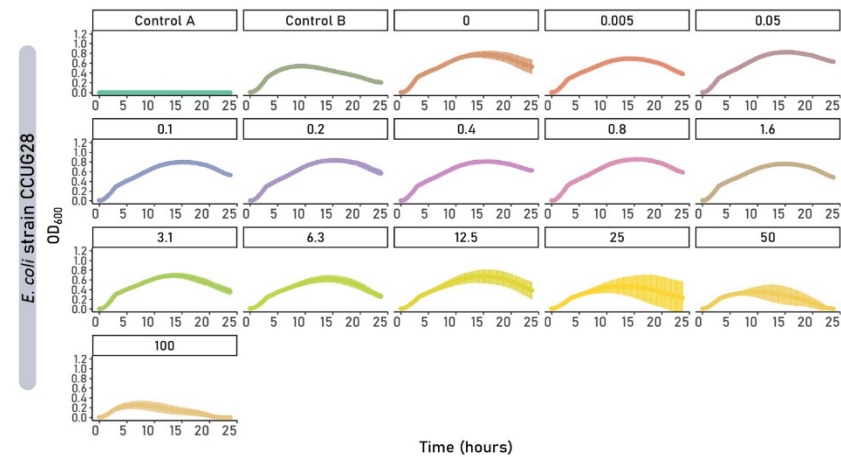
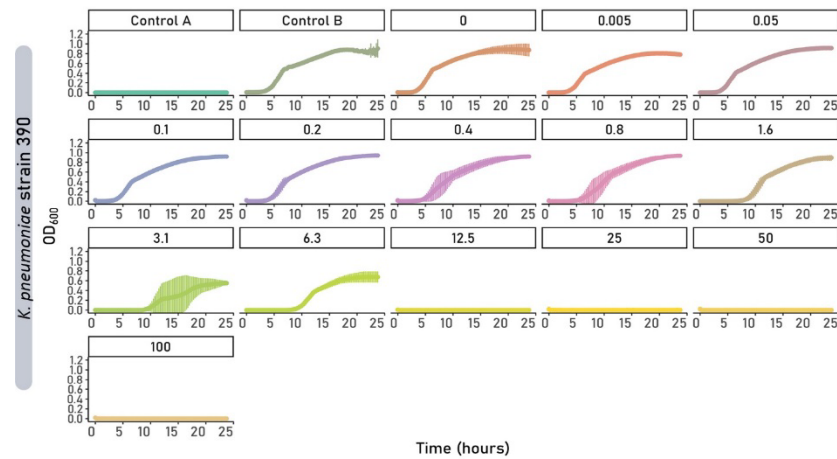


R2-K63-a

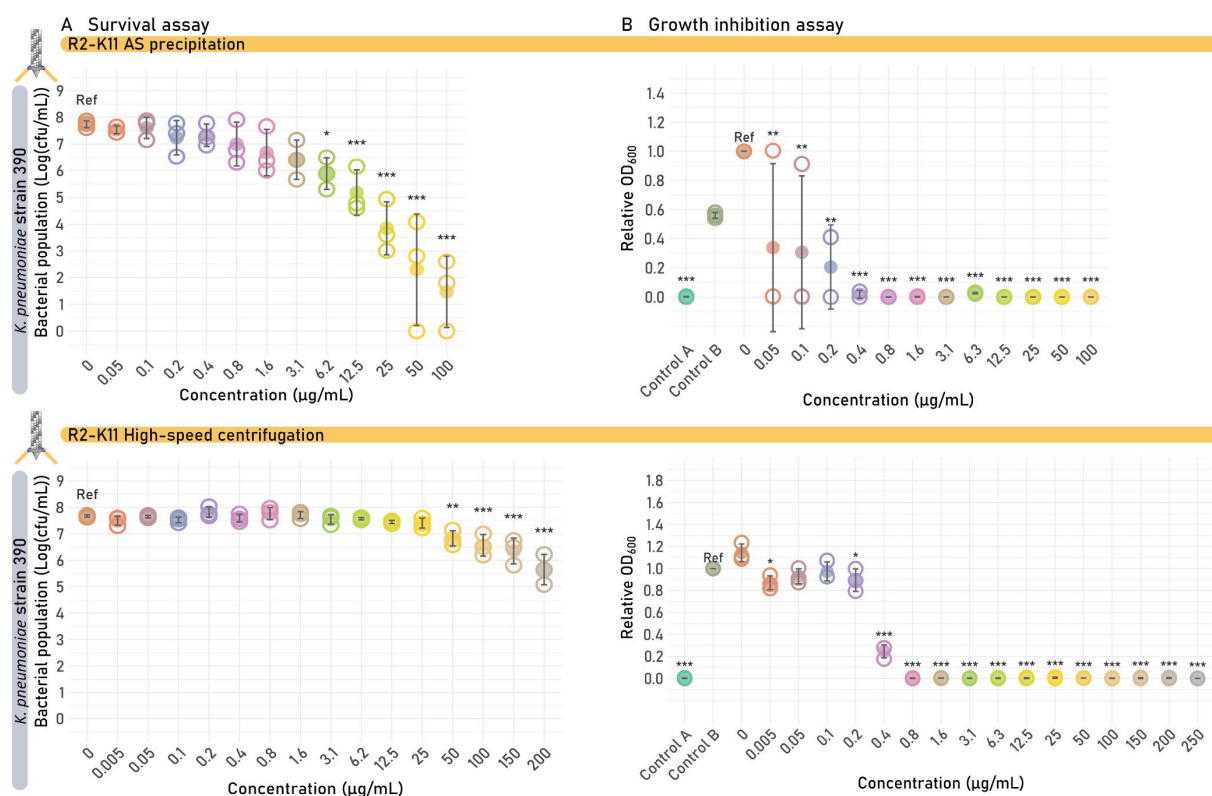


R2-K63-b





Supplementary Figure S5. 24-hour growth curves of the growth inhibition assay. The mean relative turbidity of the target strain mixed with different tailocins concentrations was monitored at 15-minute intervals over a 24-hour duration. Two additional controls were performed, one containing R2 tailocin but lacking the bacterial strain (Control A) and one containing a receptor-binding protein (RBP)-lacking mutant R2 tailocin particle (R2 Δ prf15) instead of the engineered R2 tailocin of interest (Control B).



Supplementary Figure S6. Schematic overview and experimental results of R2 tailocin R2-K11 purified by two different methods, namely AS precipitation (upper graphs) and high-speed centrifugation (lower graphs). R2-K11 was tested on its susceptible *K. pneumoniae* target strain 390, which are indicated in vertical gray colored headings on the left side of the figure. Significant differences are shown using asterisks (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$). Reference (ref) indicates the value that was used as a reference for statistical comparison (untreated sample or control B). **(A)** Results of the survival assays. The plot showed the bacterial population in function of the concentration of the added R2-K11 tailocin. The value of each biological replicate is displayed using open circles and the mean values are shown as filled circles. Values of the untreated sample were used as reference for statistical analysis. **(B)** Results of the growth inhibition assay at 8 h are shown per R2-K11 tailocin purified with AS precipitation (upper graph) and high-speed centrifugation (lower graph). The relative OD₆₀₀ of each biological replicate is displayed using open circles, and the mean relative OD₆₀₀ is shown as full circles. Two additional controls were performed, one containing R2 tailocin but lacking the bacterial strain (Control A) and one containing an RBP-lacking mutant R2 tailocin particle (R2 Δ prf15) (Control B). Both controls were added at the same concentration as the highest available R2 tailocin concentration (200-250 μ g/mL).

Supplementary Table S2. List of all bacterial strains and their origin used in this research.

Strain name	Origin	Additional information
<i>K. pneumoniae</i> strains		
390	MIT ¹	Capsular serotype K11, susceptible to phage K11 (Ando et al., 2015)
31	IP ²	Capsular serotype K31
77	UWr ³	Capsular serotype K63, susceptible to phage KP34 and KP36
486	UWr ³	Capsular serotype K63, susceptible to phage KP34 and KP36
<i>P. aeruginosa</i> strains		
PAO1	KU ⁴ , AvidBiotics	R2 tailocin producer (Williams et al., 2008)
PAO1 <i>Δprf15</i>	KU ⁴	<i>P. aeruginosa</i> PAO1 lacking the R2 tailocin tail fiber gene <i>prf15</i> (Williams et al., 2008)
wtb/CF510	DTU ⁵	Strain susceptible to PAO1 (Rau et al., 2012)
<i>E. coli</i> strains		
5	ZHAW ⁶ , UZH ⁷	Serogroup O26
A11-2162	ZHAW ⁶ , UZH ⁷	Serotype O26:H31
NVH-848	NMBU ⁸	Serotype O103:H25 (L'Abée-Lund et al., 2012)
4215/4	ZHAW ⁶ , UZH ⁷	Serogroup O103, susceptible to phage PAS7
P11-2315	ZHAW ⁶ , UZH ⁷	Serogroup O103
ECOR26	KU ⁴	Serogroup O104 (Ochman & Selander, 1984)
ECOR27	KU ⁴	Serogroup O104 (Ochman & Selander, 1984)
ECOR28	KU ⁴	Serogroup O104 (Ochman & Selander, 1984)
10-1120	ZHAW ⁶ , UZH ⁷	Serogroup O111
P10-1119	ZHAW ⁶ , UZH ⁷	Serogroup O145
A11-1581	ZHAW ⁶ , UZH ⁷	Serotype O145:H34
A11-1675	ZHAW ⁶ , UZH ⁷	Serotype O146:H21, susceptible to phage PAS61
264	ZHAW ⁶ , UZH ⁷	Serogroup O157
332	ZHAW ⁶ , UZH ⁷	Serogroup O157
584	ZHAW ⁶ , UZH ⁷	Serogroup O157
777/1	ZHAW ⁶ , UZH ⁷	Serogroup O157
2905	ZHAW ⁶ , UZH ⁷	Serogroup O157
Sakai (stx-)	UoE ⁹	Serotype O157:H7, susceptible to phage Tp10 (Dahan et al., 2004)
CAB1	UT Austin ¹⁰	Serotype O18:K1:H7, susceptible to phage K1F (Bull et al., 2010)
TOP10	Invitrogen	F- <i>mcrA</i> Δ (<i>mrr-hsdRMS-mcrBC</i>) Φ 80 <i>lacZ</i> Δ M15 Δ <i>lacX74</i> <i>recA1</i> <i>araD139</i> Δ (<i>araleu</i>)7697 <i>galU</i> <i>galK</i> <i>rpsL</i> (StrR) <i>endA1</i> <i>nupG</i>
10G	LGC Genomics	
Phages		
Escherichia phage T7	MIT ¹	Host <i>E. coli</i> strain 10G
Escherichia phage vB_EcoP_PAS7	UGent, ZHAW ⁶	Host <i>E. coli</i> strain 4215/4 (Pas et al., 2024)
Escherichia phage vB_EcoP_PAS61	UGent, ZHAW ⁶	Host <i>E. coli</i> strain A11-1675 (Pas et al., 2024)
Escherichia O157 typing phage 10 (Tp10)	UoE ⁹	Host <i>E. coli</i> strain Sakai (stx-) (Cowley et al., 2015)
Escherichia phage K1F	UT Austin ¹⁰	Host <i>E. coli</i> strains CCUG28 and CAB1 (Bull et al., 2010)
Klebsiella phage K11	MIT ¹	Host <i>K. pneumoniae</i> strain KP390 (Ando et al., 2015)

Klebsiella phage KP34	UWr ³	Host <i>K. pneumoniae</i> strains KP77 and KP486 (Drulis-Kawa et al., 2011; Maciejewska et al., 2023)
Klebsiella phage KP36	UWr ³	Host <i>K. pneumoniae</i> strains KP77 and KP486 (Kęsik-Szeloch et al., 2013)
Klebsiella phage KP32	UWr ³	Host <i>K. pneumoniae</i> strain KP271 and KP968 (Squeglia et al., 2020)

¹ Synthetic Biology Group, Massachusetts Institute of Technology (MIT), USA

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⁴ Department of Veterinary and Animal Sciences of the University of Copenhagen (KU; Denmark)

⁵ Department of Biotechnology and Biomedicine, Technical University of Denmark (DTU; Denmark)

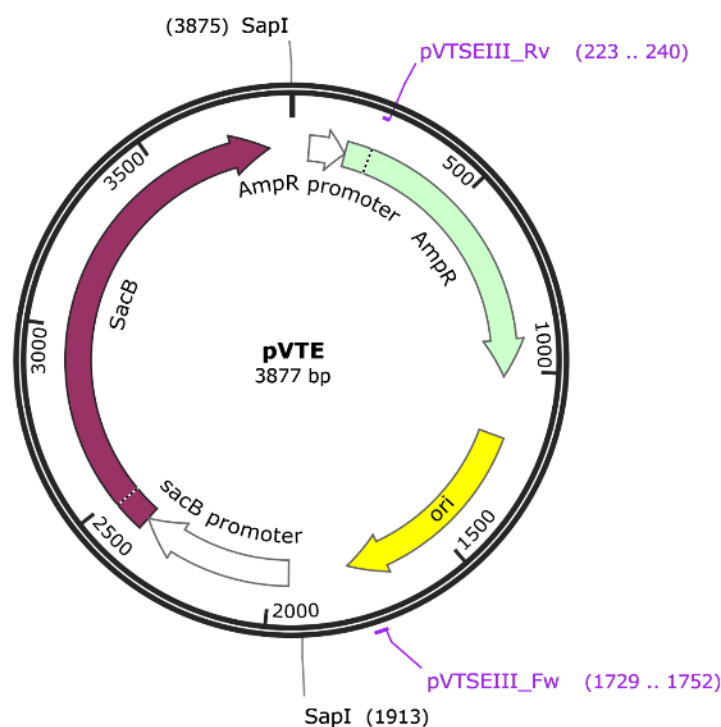
⁶ Centre for Food Safety and Quality Management of the Zurich university of applied sciences (ZHAW, Switzerland))

⁷ Institute for Food Safety and Hygiene of the University of Zurich (UZH)

⁸ Department of Paraclinical Sciences of the Norwegian University of Life Sciences (NMBU)

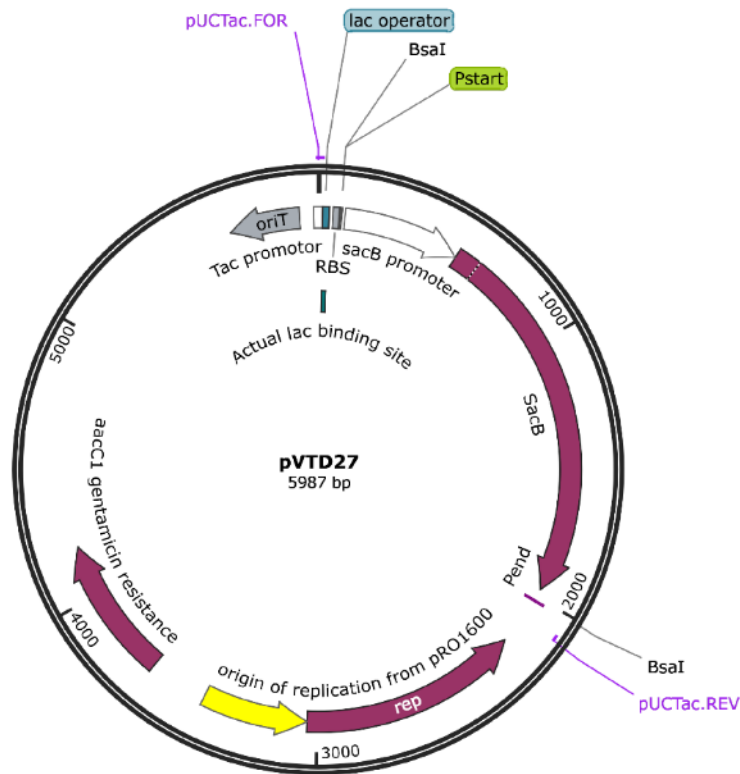
⁹ University of Edinburgh (UoE; Scotland)

¹⁰ Department of Molecular Biosciences, University of Texas at Austin (MOL, UT Austin)

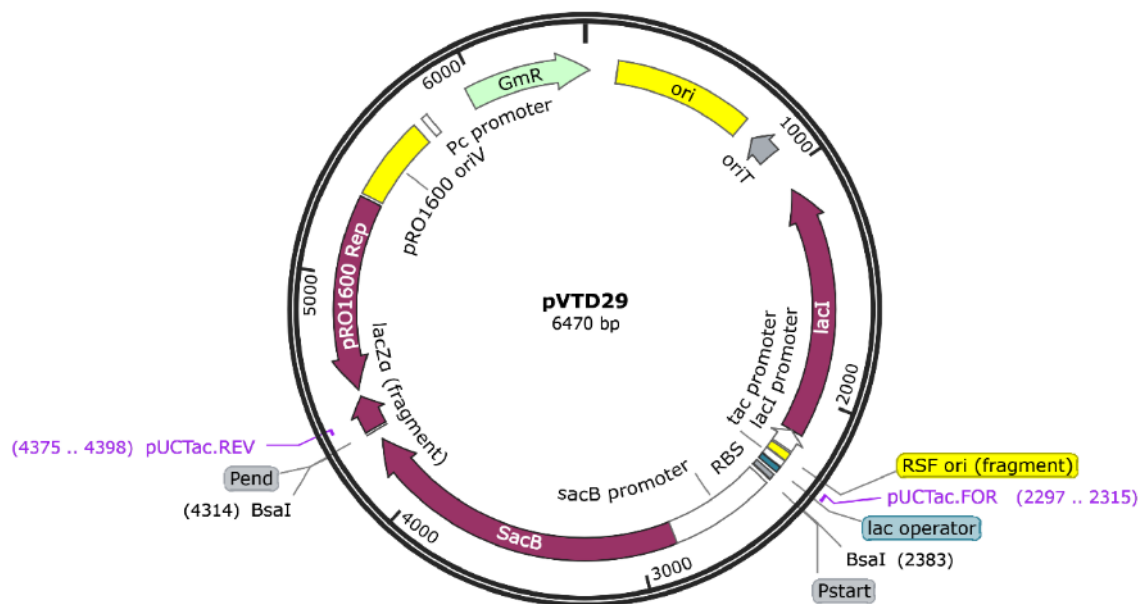


Supplementary Figure S7. Map of the pVTE cloning vector used in the VersaTile cloning step to construct tiles.

Figure obtained using Snapgene v7.1.1.



Supplementary Figure S8. Map of pVTD27 shuttle expression vector without a *lacI* repressor gene used for the VersaTile assembly reaction and expression of the receptor-binding protein in *Pseudomonas aeruginosa*. Figure obtained using Snapgene v7.1.1.



Supplementary Figure S9. Map of pVTD29 shuttle expression vector containing a *lacI* repressor gene used for the VersaTile assembly reaction and expression of the receptor-binding protein in *Pseudomonas aeruginosa*. Figure obtained using Snapgene v7.1.1.

Supplementary Table S3. Primers for tile preparation. Starting from the DNA ends, the following parts were attached: 1) a Sapl recognition (underlined) and restriction site for cloning in the pVTE vector; 2) a Bsal recognition site (underlined) for VersaTile assembly; (3) and a position-specific tag of two amino acids defining their order in the final construct. The position-specific tags used for this engineered tailocin design were P_{start} (5' ACCATG 3') – P_{mid} (position tag middle 5' GGTTC 3') for the anchor tile and P_{mid} – P_{end} (5' GGTAAT 3') for the RBD tile. These position tags are indicated in bold and restriction enzyme recognition sites (Sapl and Bsal respectively) are underlined.

Tile name	Name Primer	Primer sequence	Target DNA	Target DNA Accession number	Amplified region (aa)
Anchor-DUF3751	BB2_DUF3751_Bpil.fw	GTGGAAGACGCTAGAGGTCTC ACCATG ACGACCAATACTCCGAAATACGG	PAprf15/16	NP_249311.1	1-163
	Rv_Anchor_P2P3_2	ATAGAAGACTAGCTTGGTCTC TGAAC CTTTCTTCACCCAGTCC			
RBP-PAprf15/16	BB2_DUF3751_Bpil.fw	GTGGAAGACGCTAGAGGTCTC ACCATG ACGACCAATACTCCGAAATACGG	PAprf15/16	NP_249311.1; NP_249312.1	1-691; 1-152
	Rv_RBP_P2P7_1	GTGGAAGACTAGCTTGGTCTC ATTAC CTCTCAGGGGGTGACATAACGTCTGAGC			
RBD-PAprf15/16	BB3_PEG0prf15-16_164.fw	GTGGAAGACGCTAGAGGTCTC GGTTCA GTCTGCGGATTTCAAGG	PAprf15/16	NP_249311.1; NP_249312.1	164-691; 1-152
	Rv_RBP_P2P7_1	GTGGAAGACTAGCTTGGTCTC ATTAC CTCTCAGGGGGTGACATAACGTCTGAGC			
RBD-K11	BB3_K11gp17E.Fw	GTGGCTCTTCAAGAGGTCTC GGTTCA GCGCTACTGCCGTGTCTAATCTTCTG	K11gp17	YP_002003830.1	259-876
	BB3_K11gp17E.Rv	GTGGCTCTTCACTTGGTCTC ATTAC CTCTTATAAAACAAATGATGCTAATCTCGAGATG			
RBD-K11 (bivalent)	2E FOR Tile 2E FOR K11gp17E	GTGGCTCTTCAAGAGGTCTC GGTTCA GAGCATGACACCACTCTCTCAAG	K11gp17	YP_002003830.1	179-876
	K11gp17 Enz875 P5 REV	GTGGCTCTTCACTTGGTCTC TTCTC CTTATAAAACAAATGATGCTAATCTCGAGATGTTTC			
RBD-K31	fw_K11gp0043P3/P7	ATAGCTCTTCCAGAGGTCTC GGTTCA AACTACACCGACATGCAAGAG	K11gp0043	YP_002003831.1	2-597
	Rv_K11gp0043P3/P7	GTGGCTCTTCCCTTGGTCTC ATTAC CTTAGTTAAGGAACAGCCTAACC			
RBD-KP63-a	KP34gp57P3P7_2/630	GTGGCTCTTCAAGAGGTCTC GGTTCA GCACTCACTAAACTAGTAGATGCAG	KP34gp57	YP_003347651.1	2-630
	BB3_KP34gp57.Rv	GTGGCTCTTCACTTGGTCTC ATTAC CTCCCTAACCAAGTGAAGTTCAGATGGAGCAA			
RBD-KP63-b	BB3_KP36gp50.Fw	GTGGCTCTTCACTTGGTCTC GGTTCA AGCGCTGCGGCTGCTGCTGCGTCTGAAAATG	KP36gp50	YP_009226011.1	136-883
	BB3_KP36gp50.Rv	GTGGCTCTTCAAGAGGTCTC ATTAC CTCTTATGCCGTCAAATCTTCTAAGTGAAGATTC			
RBD-K1	K1Fgp17_E_246_P3_FOR	ATAGCTCTTCAAGAGGTCTC GGTTCA GCTAAAGGGGATGGTGTCACTGATG	K1Fgp17	YP_424959.1	246-1065
	K1Fgp17_E_1065_P7	GTAGCTCTTCACTTGGTCTC ATTAC CTCTTACTTCTGTTCAAGAGCAGAAAGTCTGGC			
RBD-K1 (bivalent)	K1Fgp17_E_246_P6_FOR	ATAGCTCTTCAAGAGGTCTC AGCGG AGCTAAAGGGGATGGTGTCACTGATG	K1Fgp17	YP_424959.1	246-1065
	K1Fgp17_E_1065_P7_REV	GTAGCTCTTCACTTGGTCTC ATTAC CTCTTACTTCTGTTCAAGAGCAGAAAGTCTGGC			
RBD-O26	BB3_RBP_O26_119_Fw	GTGGCTCTTCCAGAGGTCTC GGTTCA CTCTCTGAGCGAAGATCACTC	<i>E. coli</i> E2865 prophage tailspike	BBF60415.1	119-705
	BB3_RBP_O26_706_Rv	GTGGCTCTTCCCTTGGTCTC ATTAC CTTAAAGTTTCCCTCTGATAAG			
RBP-O103	BB3_RBP_7b_Fw	GTGGCTCTTCCAGAGGTCTC GGTTCA TCACTTGTAACTAGTACGCGAC	PAS7 tailspike	WMX18832.1	2-480

	BB3_RBP_7b_Rv	GTGGCTCTTCCCTTGGTCTC ATTAC CTTAAGCGAGTCTAGTGAATACCCAC			
RBD-O104	BB3_RBP_O104_118_Fw	ATAGCTCTTCCAGAGGTCTC GGTTC ATTACAGGGGAGCGCAGGAGTC	<i>E. coli</i> LB226692 prophage tailspike	WP_000129924.1	118-659
	BB3_RBP_O104_660_Rv	GTGGCTCTTCCCTTGGTCTC ATTAC CTTAATTGTGCTCCTTAGCAAAGCG			
RBD-O111	BB3_RBP_O111_Fw	GTGGCTCTTCCAGAGGTCTC GGTTC ACAATTAAGACAAGAGCTAGCGGGTC	<i>E. coli</i> 110512 prophage tailspike	WP_136721029.1	113-645
	BB3_RBP_O111_Rv	GTGGCTCTTCCCTTGGTCTC ATTAC CCTAGCCATCATACCAAGCATTGTTAAG			
RBD-O145	BB3_RBP_O145_118_Fw	GTGGCTCTTCCAGAGGTCTC GGTTC ATTCCCTCAGCACTTTCTCAATTAGG	<i>E. coli</i> FHI58 prophage tailspike	LM995999.1	118-675
	BB3_RBP_O145_676_Rv	GTGGCTCTTCCCTTGGTCTC ATTAC CCTATTCTGAAATATATGATCCG			
RBD-O146	BB3_RBP_61_Fw	GTGGCTCTTCCAGAGGTCTC GGTTC AGCAACAAAGAATTATGCTGATGAGC	PAS61 tailspike	WMX19002.1	224-745
	BB3_RBP_61_Rv	GTGGCTCTTCCCTTGGTCTC ATTAC CTCAGTTAGCCAGCGATACAGAC			
RBD-O157	BB3_RBP_Tp10_Fw	GTGGCTCTTCCAGAGGTCTC GGTTC AGCTACAAAAAATATGTTGATGGGGTCGC	Tp10 tailspike	AKE46458.1	216-875
	BB3_RBP_Tp10_Rv	GTGGCTCTTCCCTTGGTCTC ATTAC CTTAAATATTTCCGTTCAGCGTGCGG			
CP-KP32 (bivalent)	CP_K32_F	GTGGAAGACGCTAGAGGTCTC GGAGAAC CTCATGTTAGACAATTTCAATCAG	KP32 conserved pepide (CP)	NC 013647.1	1-35
	CP_KP32_R	ATAGAAGACGCGCTTGGTCTC CCGCT GAAGGACTCAAGGCGCGGTAATG			

Supplementary Table S4. Components and conditions for tile sequence amplification for the VersaTile cloning step using Phusion polymerase. Annealing temperature 1 (TA1) represented the optimal TA only for the matching parts of both the forward and the reverse primer. This temperature was used in the first ten cycles to ensure efficient primer annealing to the template. The Thermo Fisher Scientific TA calculator tool was used to calculate the optimal annealing temperature.

Ingredients	Volume	Conditions	Temperature (°C)	Time	Cycles
5x High-Fidelity buffer (Thermo Fisher)	10 µL	Initial denaturation	98	30 s	1
10 mM dNTPs (Thermo Fisher)	1 µL	Denaturation	98	10 s	10
Forward primer (10 µM)	2.5 µL	Annealing	TA1	25 s	
Reverse primer (10 µM)	2.5 µL	Extension	72	30 s/kb (<30s)	
Template DNA	50-100 ng	Denaturation	98	10 s	20
Phusion DNA polymerase (2 U/µL) (Thermo Fisher)	0.5 µL	Annealing	72	25 s	
Ultrapure water	variable	Extension	72	30 s/kb (≥ 30 s)	
Total volume	50 µL	Final extension	72	10 min	1

Supplementary Table S5. Components and conditions for the restriction-ligation reaction of the VersaTile cloning step.

Ingredients	Volume	Conditions	Temperature (°C)	Time	Cycles
Buffer T4 ligase (Thermo Fisher)	2 µL	Restriction	37	2 min	30
T4 DNA Ligase (5 U/µL) (Thermo Fisher)	3 µL	Ligation	22	3 min	
SapI (5 U/µL) (Thermo Fisher)	1 µL	Ligase inactivation	50	5 min	1
pVTE	25 nM	Type IIs inactivation	65	20 min	1
Tile fragment	46 nM				
Ultrapure water	variable				
Total volume	20 µL				

Supplementary Table S6. Components and conditions for the restriction-ligation reaction of the VersaTile assembly step.

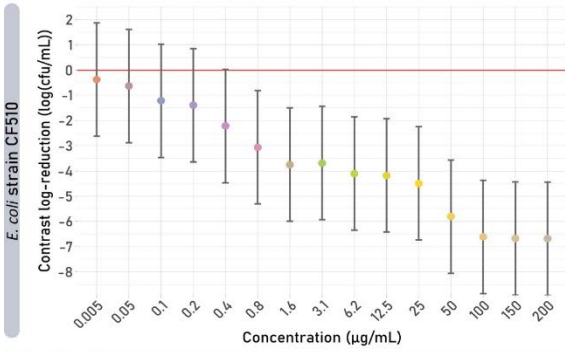
Ingredients	Volume	Conditions	Temperature (°C)	Time	Cycles
pVTD27/pVTD29 (25 nM)	1 µL	Restriction	37	5 min	25
Tile (46 nM)	1 µL/Tile	Ligation	22	5 min	
10X ligation buffer (Thermo Fisher)	2 µL	Ligase inactivation	50	5 min	1
BsaI (10 U/µL) (Thermo Fisher)	1 µL	BsaI inactivation	80	5 min	1
T4 DNA ligase (5 U/µL) (Thermo Fisher)	3 µL				
Ultrapure water	variable				
Total volume	20 µL				

Supplementary Table S7. Overview of the different wild-type and engineered R2 tailocins that were produced in this work. The receptor-binding protein (RBP) expression method, the possible nucleotide junction between anchor and receptor-binding domain (RBD), the method of purification and the expression vector that was used are indicated per construct.

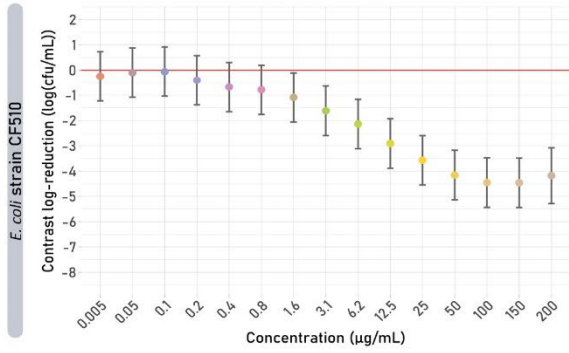
Construct name	RBD origin	RBD origin accession	Expression RBP	Interdomain junction (nt)	Purification method	Expression vector
R2-WT	<i>P. aeruginosa</i> strain PAO1	NC_002516.2	In genome	/	Both	/
R2-WT-trans	<i>P. aeruginosa</i> strain PAO1	NC_002516.2	In trans	/	Both	pVTD29
R2-WT-VT	<i>P. aeruginosa</i> strain PAO1	NC_002516.2	In trans	GGTTCA	Both	pVTD29
R2 Δ prf15	<i>P. aeruginosa</i> strain PAO1 Δ prf15	/	/	/	Both	/
R2-O26	<i>E. coli</i> strain RM10386 prophage	AP018808.1	In trans	GGTTCA	High-speed centrifugation	pVTD29
R2-O103-a	Escherichia phage vB_EcoP_PAS7	OQ921331.1	In trans	GGTTCA	High-speed centrifugation	pVTD29
R2-O103-b	Escherichia phage vB_EcoP_PAS7	OQ921331.1	In trans	CTTGGTTCA	High-speed centrifugation	pVTD27
R2-O104	<i>E. coli</i> strain LB226692 prophage	NZ_CP024992.1	In trans	GGTTCA	High-speed centrifugation	pVTD29
R2-O111	<i>E. coli</i> strain 110512 prophage	NZ_AP019761.1	In trans	GGTTCA	High-speed centrifugation	pVTD29
R2-O145-a	<i>E. coli</i> strain FHI58 prophage	LM995999.1	In trans	GGTTCA	High-speed centrifugation	pVTD29
R2-O145-b	<i>E. coli</i> strain FHI58 prophage	LM995999.1	In trans	CTTGGTTCA	High-speed centrifugation	pVTD27
R2-O146	Escherichia phage vB_EcoP_PAS61	OQ921333.1	In trans	GGTTCA	High-speed centrifugation	pVTD29
R2-O157	Escherichia phage O157 typing phage 10 (Tp10)	KP869108.1	In trans	GGTTCA	High-speed centrifugation	pVTD29
R2-K1	Escherichia phage K1F	NC_007636.1	In trans	GGTTCA	High-speed centrifugation	pVTD29
R2-K11	Klebsiella phage K11	NC_011043.1	In trans	GGTTCA	Both	pVTD29
R2-K31	Klebsiella phage K11	NC_011043.1	In trans	GGTTCA	AS precipitation	pVTD29
R2-K63-a	Klebsiella phage KP34	NC_013649.2	In trans	GGTTCA	High-speed centrifugation	pVTD29
R2-K63-b	Klebsiella phage KP36	NC_029099.1	In trans	GGTTCA	High-speed centrifugation	pVTD29
R2-K11-K1	Klebsiella phage K11/ Escherichia phage K1F	NC_011043.1 /NC_007636.1	In trans	GGTTCA	High-speed centrifugation	pVTD29

VT: VersaTile; AS: ammonium sulfate

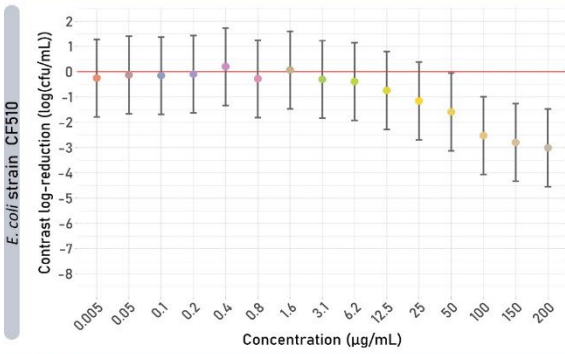
R2-WT



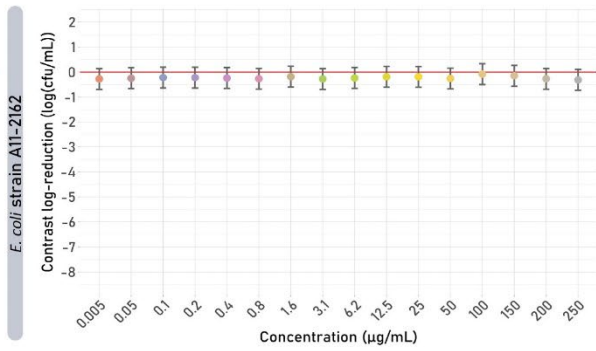
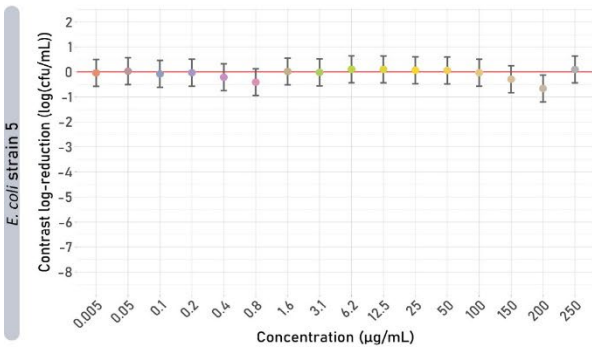
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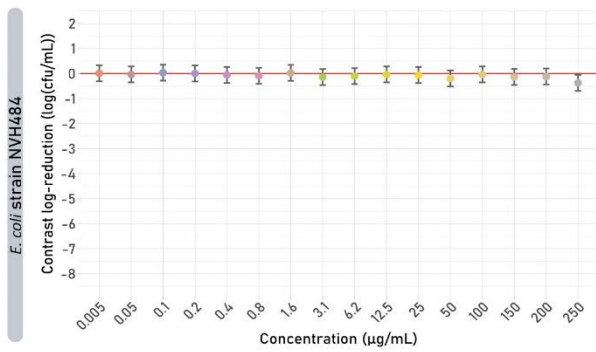
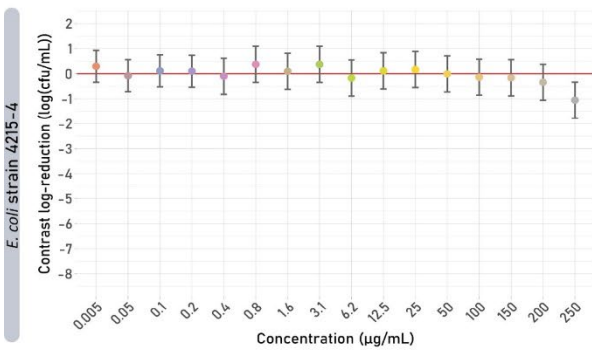
R2-WT-VT



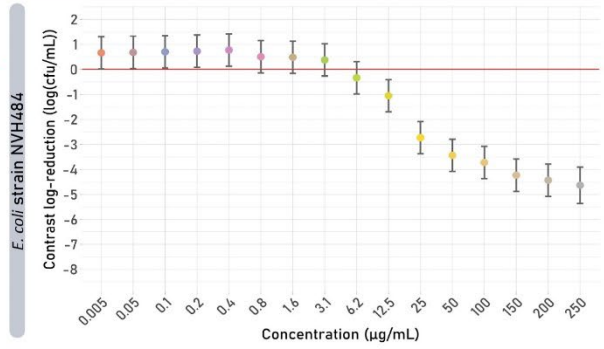
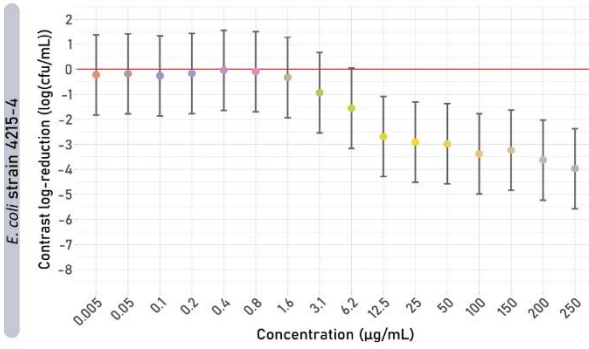
R2-026



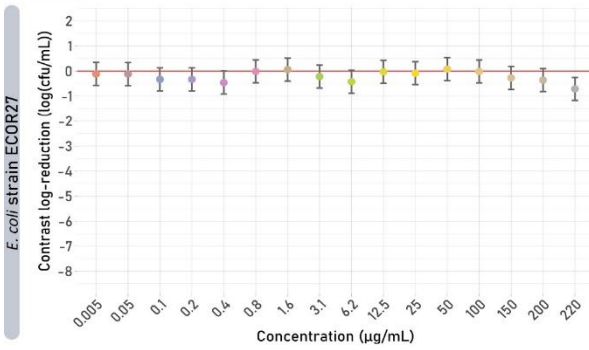
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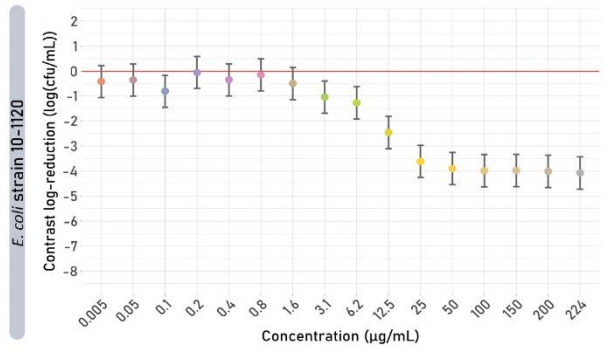
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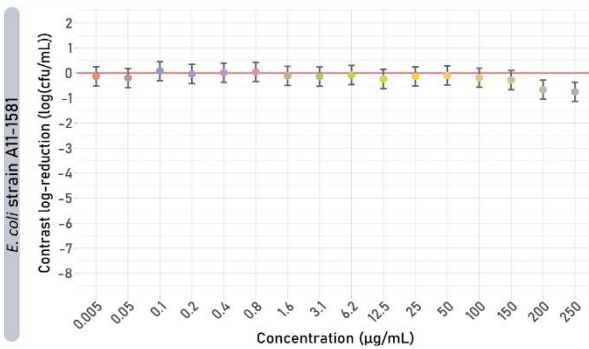
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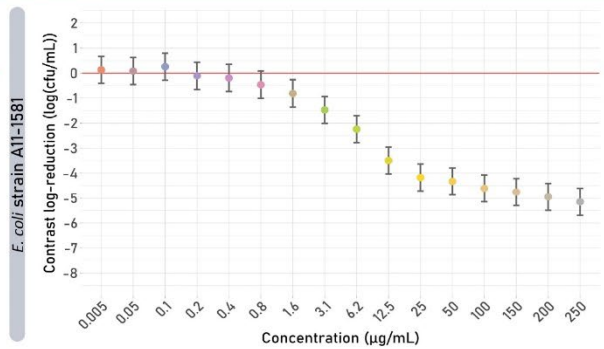
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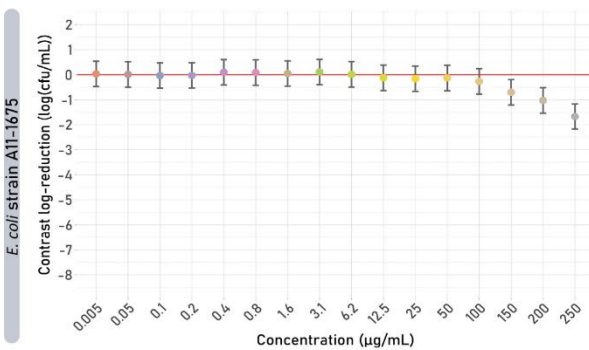
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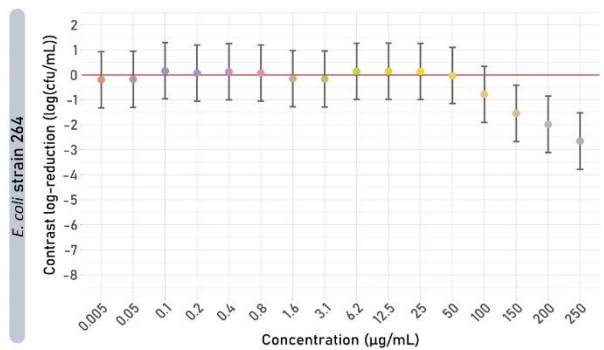
R2-0145-b



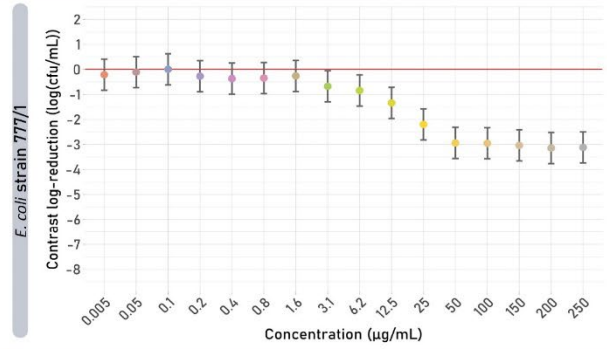
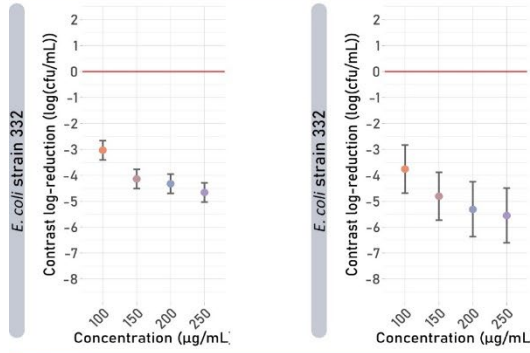
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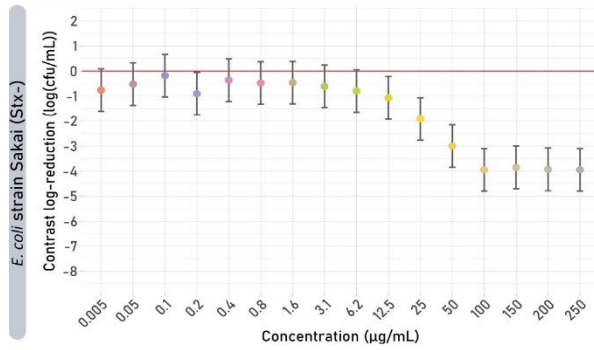
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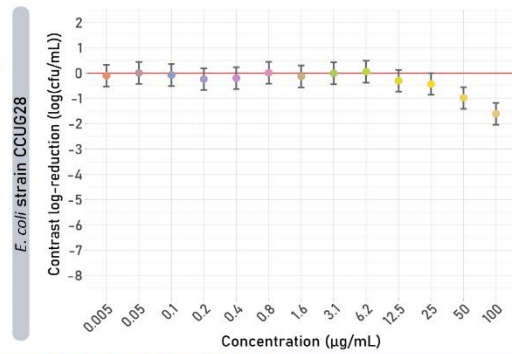
R2-0157



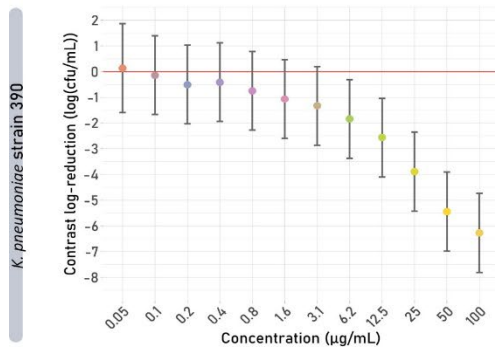
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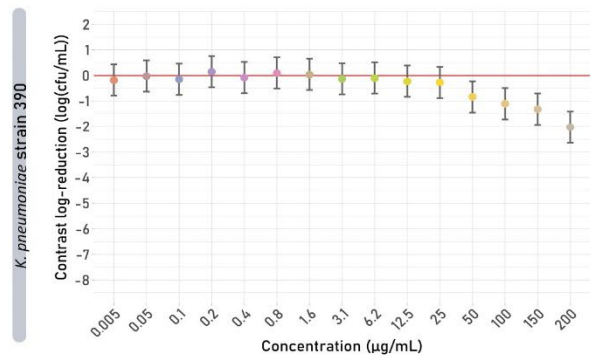
R2-K1



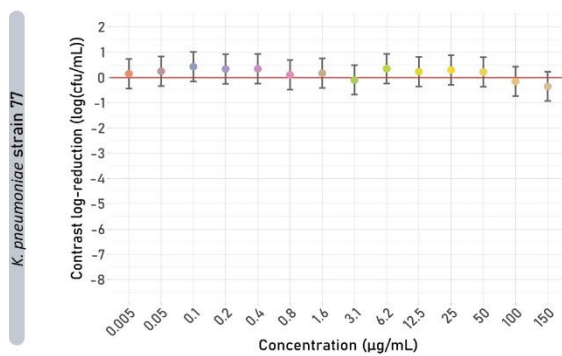
R2-K11 AS precipitation



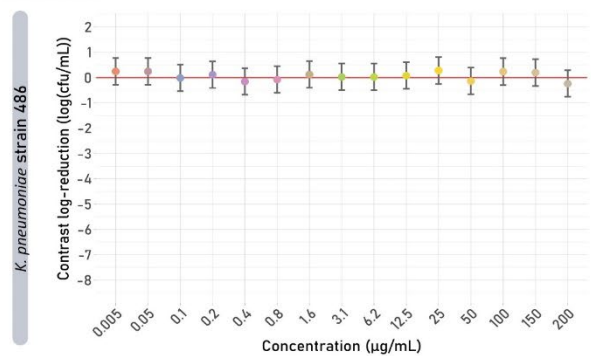
R2-K11 High-speed centrifugation

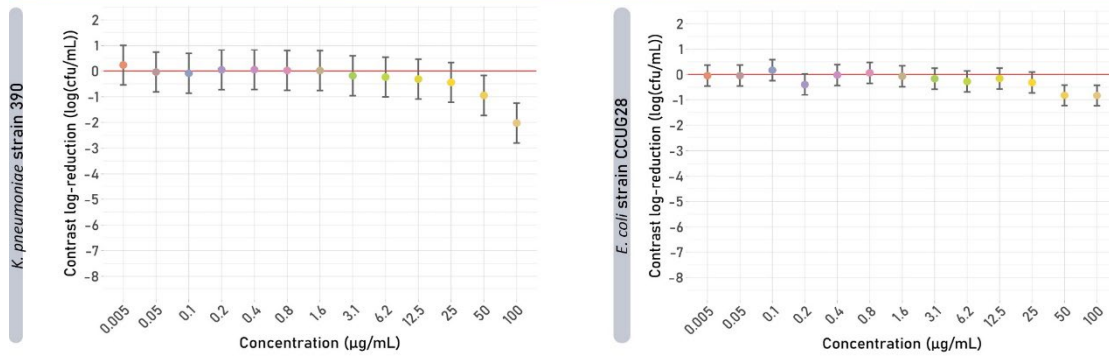


R2-K63-a



R2-K63-b





Supplementary Figure S10. Statistical analysis of the survival assay. A linear mixed model with random intercept per biological replicate was fitted to the data. The bacterial log-reduction compared to the zero concentration, or the contrast in short, was plotted in function of the concentration of the produced R2 tailocin with joint confidence intervals. All concentrations for which the confidence interval lies below the zero line indicate a significant bacterial log-reduction at that concentration.