Supplementary Tables.

Table S1. Statistical values.

Figure 4a	*p* value
HDR	0.0009
HD+1	< 0.0001
HD+2	< 0.0001
HD+3	0.0003
HD+4	< 0.0001
HD+5	< 0.0001
HD+6	0.0002
HD+7	0.0006
HD+8	0.0006
HD+9	0.0024
HD+10	0.0009
HD+11	0.0029
HD+12	0.0266
Figure 4b	***p* value**
HDR	0.0002
HD+1	< 0.0001
HD+2	0.0002
HD+3	0.0001
HD+4	0.0014
HD+5	< 0.0001
HD+6	0.0001
HD+7	0.0023
HD+8	0.0005
HD+9	0.0081
HD+10	0.0041

Figure 5a. Lateral transfer	*p* value
0-30	0.9974
0-60	0.0899
0-90	< 0.0001
0-120	< 0.0001
0-150	< 0.0001
0-180	< 0.0001
0-210	< 0.0001
0-240	< 0.0001
0- -	< 0.0001
Figure 5b. Phage titre	***p* value**
0-30	0.9975
0-60	0.9920
0-90	0.9101
0-120	0.1489
0-150	0.0017

0-180	< 0.0001
0-210	< 0.0001
0-240	< 0.0001
0- -	< 0.0001

Figure S5. qPCR EXCISION	***p* value**
P22 0-30	>0.9999
P22 0-60	0,1118
P22 0-90	0,0405
P22 ts 0-30	0,1916
P22 ts 0-60	0,6757
P22 ts 0-90	>0.9999
P22 Δ*pri* 0-30	>0.9999
P22 Δ*pri* 0-60	0,7658
P22 Δ*pri* 0-90	0,2902
ES18 0-60	0,6209
ES18 0-90	0,0258
ES18 0-120	0,0033

Figure S5. qPCR CIRCULARISATION	**p value**
P22 0-30	>0.9999
P22 0-60	0,5923
P22 0-90	<0.0001
P22 ts 0-30	0,9749
P22 ts 0-60	0,3107
P22 ts 0-90	0,8334
ES18 0-60	0,9989
ES18 0-90	0,0003
ES18 0-120	<0.0001

Figure S7	***p* value**
HD+1	0.1760
HD+2	0.2911
HD+3	0.0741
HD+4	0.8390
HD+5	0.1275
HD+6	0.4479
HD+7	0.5348
HD+8	0.0677
HD+9	0.6282
HD+10	0.9924
HD+11	0.1713
HD+12	0.7911

Figure S8a	***p* value**
HD+1	0.0207
HD+2	0.0131
Figure S8b	***p* value**
HD+1	0.0013
HD+2	0.0009

Figure S9	***p* value**
HD+1	0.0008
HD+2	0.0023
HD+4	0.0082
*tet*M^{G}	0.9364

Figure S10. P22 LT	***p* value**
wt-Δ*int*	0.0003
wt-Δ*xis*	0.0002
wt-Δ*int-xis*	0.0002
Figure S10. P22 titre	***p* value**
wt-Δ*int*	< 0.0001
wt-Δ*xis*	< 0.0001
wt-Δ*int-xis*	< 0.0001

Figure S10. ES18 LT	***p* value**
wt-Δ*int*	0.0136
wt-Δ*xis*	0.0335
wt-Δ*int-xis*	0.0434
Figure S10. ES18 titre	***p* value**
wt-Δ*int*	< 0.0001
wt-Δ*xis*	< 0.0001
wt-Δ*int-xis*	< 0.0001

Table S2. Strains used in this study.

Strain	Description	Reference
E. coli DC10B	K-12-derivative cloning strain; *dam*$^+$ *Ddcm DhsdRMS endA1 recA1*	[1]
VE18590	*E. faecalis* V583 derivative. Non-lysogenic, EfCIV583-negative.	[2]
VE18562	*E. faecalis* V583 derivative. fp1 lysogen, EfCIV583-negative.	[2]
JP18938	LT2 ΔFels-1 ΔGifsy-2 ΔGifsy-1 ΔFels-2	This work
JP22117	SV1208 ΔFels-1	This work
JP18983	JP18938 P22 lysogen	This work
JP18985	JP18938 ES18 lysogen	This work
JP22119	JP22117 P22 tsc_2*29* lysogen	This work
JP20460	JP18938 Δ*rfb*P-*rfb*B::*Km*R	This work
JP20590	JP18938 P22 lysogen Δ*int*	This work
JP20591	JP18938 P22 lysogen Δ*xis*	This work
JP20592	JP18938 P22 lysogen Δ*int-xis*	This work
JP20593	JP18938 P22 lysogen Δ*orf12*	This work
JP20595	JP18938 ES18 lysogen Δ*int*	This work
JP20596	JP18938 ES18 lysogen Δ*xis*	This work
JP20597	JP18938 ES18 lysogen Δ*int-xis*	This work
JP19020	JP18938 Δ*att*B P22	This work
JP22118	JP22117 Δ*att*B P22	This work
JP19086	JP19020 *gpt*ΩTetA (6 kb upstream P22 *att*B)	This work
JP19087	JP19020 STM0331ΩTetA (6 kb downstream P22 *att*B, HF1)	This work
JP19088	JP19020 *prp*RΩTetA (47 kb downstream P22 *att*B, HF2)	This work
JP19089	JP19020 *mal*ZΩTetA (88 kb downstream P22 *att*B, HF3)	This work
JP19090	JP19020 *yaj*GΩTetA (130 kb downstream P22 *att*B, HF4)	This work
JP19091	JP19020 *yba*NΩTetA (170 kb downstream P22 *att*B, HF5)	This work
JP19092	JP19020 *ybb*VΩTetA (213 kb downstream P22 *att*B, HF6)	This work
JP19093	JP19020 STM0557ΩTetA (246 kb downstream P22 *att*B, HF7)	This work
JP19094	JP19020 *ahp*FΩTetA (304 kb downstream P22 *att*B, HF8)	This work
JP19095	JP19020 *ybe*LΩTetA (348 kb downstream P22 *att*B, HF9)	This work
JP19096	JP19020 STM0699ΩTetA (392 kb downstream P22 *att*B, HF10)	This work
JP19097	JP19020 *nei*ΩTetA (425 kb downstream P22 *att*B, HF11)	This work
JP19098	JP19020 *mod*FΩTetA (474 kb downstream P22 *att*B, HF12)	This work
JP20469	JP18983 *gpt*ΩTetA (6 kb upstream P22 *att*B)	This work
JP20470	JP18983 STM0331ΩTetA (6 kb downstream P22 *att*B, HF1)	This work
JP20471	JP18983 *prp*RΩTetA (47 kb downstream P22 *att*B, HF2)	This work
JP20472	JP18983 *mal*ZΩTetA (88 kb downstream P22 *att*B, HF3)	This work
JP20473	JP18983 *yaj*GΩTetA (130 kb downstream P22 *att*B, HF4)	This work
JP20474	JP18983 *yba*NΩTetA (170 kb downstream P22 *att*B, HF5)	This work
JP20475	JP18983 *ybb*VΩTetA (213 kb downstream P22 *att*B, HF6)	This work
JP20476	JP18983 STM0557ΩTetA (246 kb downstream P22 *att*B, HF7)	This work
JP20477	JP18983 *ahp*FΩTetA (304 kb downstream P22 *att*B, HF8)	This work
JP20478	JP18983 *ybe*LΩTetA (348 kb downstream P22 *att*B, HF9)	This work
JP20479	JP18983 STM0699ΩTetA (392 kb downstream P22 *att*B, HF10)	This work
JP20480	JP18983 *nei*ΩTetA (425 kb downstream P22 *att*B, HF11)	This work
JP20481	JP18983 *mod*FΩTetA (474 kb downstream P22 *att*B, HF12)	This work
JP21928	JP22118 *gpt*ΩTetA (6 kb downstream P22 *att*B)	This work
JP21929	JP22118 *prp*RΩTetA (47 kb downstream P22 *att*B)	This work
JP21922	JP22119 *gpt*ΩTetA (6 kb downstream P22 *att*B)	This work
JP21923	JP22119 *prp*RΩTetA (47 kb downstream P22 *att*B)	This work
JP20482	JP18985 *gpt*ΩTetA (6 kb upstream P22 *att*B)	This work
JP20483	JP18985 STM0331ΩTetA (6 kb downstream P22 *att*B, HF1)	This work
JP20484	JP18985 *prp*RΩTetA (47 kb downstream P22 *att*B, HF2)	This work
JP20485	JP18985 *mal*ZΩTetA (88 kb downstream P22 *att*B, HF3)	This work
JP20487	JP18985 *yba*NΩTetA (170 kb downstream P22 *att*B, HF4)	This work
JP20488	JP18985 *ybb*VΩTetA (213 kb downstream P22 *att*B, HF5)	This work
JP20489	JP18985 STM0557ΩTetA (246 kb downstream P22 *att*B, HF6)	This work
JP20490	JP18985 *ahp*FΩTetA (304 kb downstream P22 *att*B, HF7)	This work
JP20491	JP18985 *ybe*LΩTetA (348 kb downstream P22 *att*B, HF8)	This work
JP20493	JP18985 *nei*ΩTetA (425 kb downstream P22 *att*B, HF9)	This work
JP20494	JP18985 *mod*FΩTetA (474 kb downstream P22 *att*B, HF10)	This work
JP19438	JP20470 Δ*rfb*P-*rfb*B::*Km*R	This work
JP19439	JP20471 Δ*rfb*P-*rfb*B::*Km*R	This work
JP19440	JP20472 Δ*rfb*P-*rfb*B::*Km*R	This work
JP19441	JP20473 Δ*rfb*P-*rfb*B::*Km*R	This work

JP19442	JP20474 Δ*rfb*P-*rfb*B::*Km*R	This work
JP19443	JP20475 Δ*rfb*P-*rfb*B::*Km*R	This work
JP19444	JP20476 Δ*rfb*P-*rfb*B::*Km*R	This work
JP19445	JP20477 Δ*rfb*P-*rfb*B::*Km*R	This work
JP19446	JP20478 Δ*rfb*P-*rfb*B::*Km*R	This work
JP19447	JP20479 Δ*rfb*P-*rfb*B::*Km*R	This work
JP19448	JP20480 Δ*rfb*P-*rfb*B::*Km*R	This work
JP19449	JP20481 Δ*rfb*P-*rfb*B::*Km*R	This work
JP21422	JP19020 Cat marker 6 kb downstream P22 *att*B + TetA marker 6 kb upstream P22 *att*B	This work
JP21423	JP19020 Cat marker 6 kb downstream P22 *att*B + TetA marker 10 kb up downstream P22 *att*B	This work
JP21424	JP19020 Cat marker 6 kb downstream P22 *att*B + TetA marker 29 kb up downstream P22 *att*B	This work
JP21425	JP19020 Cat marker 6 kb downstream P22 *att*B + TetA marker 32 kb up downstream P22 *att*B	This work
JP21426	JP19020 Cat marker 10 kb downstream P22 *att*B + TetA marker 29 kb upstream P22 *att*B	This work
JP21427	JP19020 Cat marker 10 kb downstream P22 *att*B + TetA marker 32 kb upstream P22 *att*B	This work
JP21428	JP19020 Cat marker 15.6 kb downstream P22 *att*B + TetA marker 29 kb upstream P22 *att*B	This work
JP21429	JP19020 Cat marker 15.6 kb downstream P22 *att*B + TetA marker 32 kb upstream P22 *att*B	This work
JP21430	JP19020 Cat marker 29 kb downstream P22 *att*B + TetA marker 32 kb upstream P22 *att*B	This work
JP21431	JP18983 Cat marker 6 kb downstream P22 *att*B + TetA marker 6 kb upstream P22 *att*B	This work
JP21432	JP18983 Cat marker 6 kb downstream P22 *att*B + TetA marker 10 kb up downstream P22 *att*B	This work
JP21433	JP18983 Cat marker 6 kb downstream P22 *att*B + TetA marker 29 kb up downstream P22 *att*B	This work
JP21434	JP18983 Cat marker 6 kb downstream P22 *att*B + TetA marker 32 kb up downstream P22 *att*B	This work
JP21435	JP18983 Cat marker 10 kb downstream P22 *att*B + TetA marker 29 kb upstream P22 *att*B	This work
JP21436	JP18983 Cat marker 10 kb downstream P22 *att*B + TetA marker 32 kb upstream P22 *att*B	This work
JP21437	JP18983 Cat marker 15.6 kb downstream P22 *att*B + TetA marker 29 kb upstream P22 *att*B	This work
JP21438	JP18983 Cat marker 15.6 kb downstream P22 *att*B + TetA marker 32 kb upstream P22 *att*B	This work
JP21439	JP18983 Cat marker 29 kb downstream P22 *att*B + TetA marker 32 kb upstream P22 *att*B	This work
JP20600	JP20590 *prp*RΩTetA (47 kb downstream P22 *att*B, HF2)	This work
JP20601	JP20591 *prp*RΩTetA (47 kb downstream P22 *att*B, HF2)	This work
JP20602	JP20592 *prp*RΩTetA (47 kb downstream P22 *att*B, HF2)	This work
JP20605	JP20595 *prp*RΩTetA (47 kb downstream P22 *att*B, HF2)	This work
JP20606	JP20596 *prp*RΩTetA (47 kb downstream P22 *att*B, HF2)	This work
JP20607	JP20597 *prp*RΩTetA (47 kb downstream P22 *att*B, HF2)	This work
JP20757	JP20602 pJP2534	This work
JP21704	*E. coli* DC10B pJP2563	This work
JP21747	*E. coli* DC10B pJP2564	This work
JP21748	*E. coli* DC10B pJP2565	This work
JP21896	*E. coli* DC10B pJP2566	This work
JP21706	*E. coli* DC10B pJP2568	This work
JP21767	VE18590 *celA*Ω*tet*M (12.3 kb upstream of pp1 *att*B)	This work
JP21855	VE18590 EF0370Ω*tetM* (12.3 kb downstream of pp1 *att*B, HF1)	This work
JP22112	VE18590 EF0394Ω*tetM* (40.7 kb downstream of pp1 *att*B, HF2)	This work
JP22021	VE18590 EF0480Ω*tetM* (121.1 kb downstream of pp1 *att*B, HF4)	This work
JP21771	VE18590 EF2276Ω*tetM* (1.87 Mb downstream of pp1 *att*B)	This work
JP21768	VE18562 *celA*Ω*tetM* (12.3 kb upstream of pp1 *att*B)	This work
JP21856	VE18562 EF0370Ω*tetM* (12.3 kb downstream of pp1 *att*B, HF1)	This work
JP21857	VE18562 EF0394Ω*tetM* (40.7 kb downstream of pp1 *att*B, HF2)	This work
JP22110	VE18562 EF0480Ω*tetM* (121.1 kb downstream of pp1 *att*B, HF4)	This work

JP21772	VE18562 EF2276Ω*tetM* (1.87 Mb downstream of pp1 *att*B)	This work

Table S3. Oligonucleotides used in this study.

Mutagenesis	Primers	Sequence (5'-3')
LT2 ΔFels-1	**LT2-AFels1-1m**	GGGGCACTCCTGGGGCAGTAGATGCCAGTTGTTGATTCAG TATATCTACTGTGTAGGCTGGAGCTGCTTCG
	LT2-AFels1-2c	GGCATCATACTGTACACTGTCATATGCCATATATTTAAACGC TAAAAGGGCATATGAATATCCTCCTTA
LT2 ΔGifsy-2	**LT2-AGifsy2-13m**	CGCTGGAGTATACCTTGTTTAGCGATTTATTGAACCCCGATC ACACCTGTGTAGGCTGGAGCTGCTTCG
	LT2-AGifsy2-14c	GCAAATCGCGCTACGCAGAATGTTCATCTTTTCAGGCACAA ACGGCCGGTCCATATGAATATCCTCCTTAG
LT2 ΔGifsy-1	**LT2-AGifsy1-1m**	CAAGTAACGAGGCGACATCAAACCTTGAGTTATTAAATAAAT GGAGAATAGTGTGTAGGCTGGAGCTGCTTCG
	LT2-AGifsy1-2c	GTCCCTGATATGGGCGTGCAGGGTCGGTGAACTCCGTCAG GCTGAAGCATATGAATATCCTCCTTA
LT2 ΔFels-2	**LT2-AFels2-1m**	CTTCAAGCTCCATGCTGGTCGTAAATCCATTATCCGGGCTG ACAGAATGCTGTGTAGGCTGGAGCTGCTTCG
	LT2-AFels2-2c	TTCCTTGAGCATCTTCGGGCGGATCTCGGTAACGGGTGTGT TACCCAGAGCATATGAATATCCTCCTTA
LT2 Δ*att*B P22	**LT2-AattB-1m**	CCTTCTACCCCGTGATTCACCCGCGTGAACACACCCTTCTC ATGTGTAGGCTGGAGCTGCTTCG
	LT2-AattB-2c	GGATACTGCTTATGTTTTGCTAGTTGTGTACCCGAGAGTGTA CCCGGTCCATATGAATATCCTCCTTAG
LT2 Δ*rfb*P-*rfb*B P22	**LT2-ArfbP-1m**	TTTTACGCAGGCTAATTTATACAATTATTATTCAGTACTTCTC GGTAAGCTTGTGTAGGCTGGAGCTGCTTCG
	LT2-ArfbB-2c	TTTATTGGCAAATTAAATACCACATTAAATACGCCTTATGGA ATAGAAAACATATGAATATCCTCCTTA
***tet*A marker 6 kb upstream P22 *att*B**	**LT2-tetA-11m**	TCAGACACGATAAGTCTCCTTGGCGGTGGTCTGAAAAACGT TCTTACAGGCCACAGGGTTCGCTGTTAATCACTTTACTT
	LT2-tetA-12c	ACCCGCAGGCGGAGAAAGTGGTATTCTCAGTCGCATCTCGT AAGAGTTATCAATTCCGCTGGTTATCAAGAGGGTCATTA
***tet*A marker 6 kb downstream P22 *att*B**	**LT2-tetA-3m**	GGTTGCGCATTATTCACGGCGGCCGTGGCTAAAACGCCCT GCATCCCCGCGCGGTTAAGGCGCTGTTAATCACTTTACTT
	LT2-tetA-4c	AATGCCGTAGCGTTTGCCACCGTTCACAAGATATTGAACCA GTTTCATTGCAGTCTTCTTGGTTATCAAGAGGGTCATTA
***tet*A marker 47 kb downstream P22 *att*B**	**LT2-tetA-6m**	ATTGCATCATAAAAATACCCGCCTGGGTTTCCGGCGGGTAT ATTTATTCCTGGCAACCGGCGCTGTTAATCACTTTACTT
	LT2-tetA-7c	GCGTCCTGAATGCCTGATGGCGCTACGTTTACTGCAGGCC GTCATCCGGCAAAACGGATGGGTTATCAAGAGGGTCATTA
***tet*A marker 88 kb downstream P22 *att*B**	**LT2-tetA-9m**	GCGAGGGAAGACTTAGTTTACCCGCGATTTCCGCGACGGT ATGGATGAATCGTTGAGGCGCGCTGTTAATCACTTTACTT
	LT2-tetA-10c	CATTGATAAAAGGCTCGCGAATGCGAGCCTTTTTTATGCGT AAGGTGTTACGAACCACCTGGTTATCAAGAGGGTCATTA
***tet*A marker 130 kb downstream P22 *att*B**	**LT2-tetA-13m**	CGTAAATATTGACTTGACATAGGCATCTACAGACCCGGCTT CTGCCGGGTCTTGTTGTTGCGCTGTTAATCACTTTACTT
	LT2-tetA-14c	GCTGATATGTCTCAGGACACCAGTATTCACGATTTTATCAAG CAAAACGCCCGTTAATAGGGTTATCAAGAGGGTCATTA
***tet*A marker 170 kb downstream P22 *att*B**	**LT2-tetA-15m**	CTGCTGATTTTTATGTGGCGGATACCGGTGATTGATGAAAA GCAACAAAAGCGCTGAAGCCGCTGTTAATCACTTTACTT
	LT2-tetA-16c	TGTGCTCGAAAACGGTCGAATTTACTGGCTGTGAACGACAA TTGCAACAGCGATTCGTGTGGTTATCAAGAGGGTCATTA
***tet*A marker 213 kb downstream P22 *att*B**	**LT2-tetA-17m**	TCACTTTATATAAAAAGAAAGCAACGCAACGTATTGCTTCAG CTTAAAAATAATTATATCCGCTGTTAATCACTTTACTT
	LT2-tetA-18c	GATAACGTTGTTATGGGTTTAGCTATGAGGAACAAATAAATA TAAAATAAAGAATTAGTAGGTTATCAAGAGGGTCATTA
***tet*A marker 246 kb**	**LT2-tetA-19m**	TGTAGGAATCCCCGCCGCCCGTTACCCATTGGTGGCGGGG AACATTAATTATACATGAATCGCTGTTAATCACTTTACTT

downstream P22 *att*B	**LT2-tetA-20c**	CATGGGACGTTAGAACAGGGGCGAGGACAAATGAGAATATT ACGGAAATAATTAAATAACGGTTATCAAGAGGGTCATTA
***tet*A marker 304 kb**	**LT2-tetA-25m**	GCCTTTGATTATCTGATTCGCACCAAAATCGCATAAAAAGAA GTAAGCACACCTGCAAGGCGCTGTTAATCACTTTACTT
downstream P22 *att*B	**LT2-tetA-26c**	AAACACCGCAGGCCCGAATAGCTTACACTATCGGGCCATTT ACGATGGCCAGTTAACTGGGGTTATCAAGAGGGTCATTA
***tet*A marker 348 kb**	**LT2-tetA-27m**	GTGTGGCCACGATCAGTTCCAGCGCAGGCCGTTTGAGCCG TAAGGTTCATTATCGTGAGCCGCTGTTAATCACTTTACTT
downstream P22 *att*B	**LT2-tetA-28c**	GAAGAGATCCGCAAACTGGAAGCCCAACTGCACGCTTAACA TGGCGGACGGCAACCGTCCGGTTATCAAGAGGGTCATTA
***tet*A marker 392 kb**	**LT2-tetA-21m**	TGAGAAAGAAGCCGTTGAAATCGTTAGCGAAGTATTGAAAA ACGCCTGATGGGCGATATGCGCTGTTAATCACTTTACTT
downstream P22 *att*B	**LT2-tetA-22c**	AGGCCTGATAAGCGCAGCGCCGTTAATACAAAAAAGGAGC CGTAAGGCTCCTTTTTCTTCGGTTATCAAGAGGGTCATTA
***tet*A marker 425 kb**	**LT2-tetA-29m**	CGCATTGCCAGAAATAGCCGGAACCGACATCGGCGAGCGG CTATTGCCTGATGGCGCGACCGCTGTTAATCACTTTACTT
downstream P22 *att*B	**LT2-tetA-30c**	AGCAGTGGCCCGTTTTATATCAACCTATGCGCCCAAACAAC CTTTGTAGGGCTGATAAGCGGTTATCAAGAGGGTCATTA
***tet*A marker 474 kb**	**LT2-tetA-23m**	TATCGACCGTTATTATTATTCTTAATAAAAGGAGAGTGGTTC CAGAATGGCGCGCGGCGCCGCTGTTAATCACTTTACTT
downstream P22 *att*B	**LT2-tetA-24c**	AGGGCGAACGTTATAAGTACGTTTCCGGACGATGCAATAAT TAAATGTATTATCAGAATGGGTTATCAAGAGGGTCATTA
***cat* marker 6 kb**	**LT2-STM0331-cat-1m**	GGTTGCGCATTATTCACGGCGGCCGTGGCTAAAACGCCCT GCATCCCCGCGCGGTTAAGGGGCGCGCCTACCTGTGACGG
downstream P22 *att*B	**LT2-STM0331-cat-2c**	AATGCCGTAGCGTTTGCCACCGTTCACAAGATATTGAACCA GTTTCATTGCAGTCTTCTTGGAATAGGAACTTCATTTAAATG GC
***tet*A marker 10 kb**	**LT2-stbE-tetA-2c**	CTCCTGAGTTTTGATGGGAAATATTCAGGGATACGATCGTAT CCCTGAGGGGTTATCAAGAGGGTCATTA
downstream P22 *att*B	**LT2-stbE-tetA-1m**	ATCAGCTTAATGCTATTTTTGACAATGGTACATGCTGAGTCT GGCTTGCGCTGTTAATCACTTTACTT
***tet*A marker 29 kb**	**LT2-STM0352-tetA-1m**	CGTTTCCCTCTTTACCGCAGCGTGTCGGCCATTCCGCAACG CTGCCGCAGCGCTGTTAATCACTTTACTT
downstream P22 *att*B	**LT2-STM0352-tetA-2c**	CCCGTCAGGAACGCTTGACCTTTCCTTCGTTGTAACGCCTA GCCTTTGCCGGTTATCAAGAGGGTCATTA
***tet*A marker 32 kb**	**LT2-STM0355-tetA-1m**	CCGGTTTCCCGCCGCGCGAGAGGTAATCGTCAATGGCGAT CGGTCTGGCGCGCTGTTAATCACTTTACTT
downstream P22 *att*B	**LT2-STM0355-tetA-2c**	CTCACGCAAAGAAAAGCGAAGCGTGGCTAGCGTATCGCGA CCGGCCTGTGGTTATCAAGAGGGTCATTA
***cat* marker 10 kb**	**LT2-stbE-cat-1m**	ATCAGCTTAATGCTATTTTTGACAATGGTACATGCTGAGTCT GGCTTGGGCGCGCCTACCTGTGACGG
downstream P22 *att*B	**LT2-stbE-cat-2c**	CTCCTGAGTTTTGATGGGAAATATTCAGGGATACGATCGTAT CCCTGAGGGGAATAGGAACTTCATTTAAATGGC
***cat* marker 15.6 kb**	**LT2-stbB-cat-1m**	CAACTCCCTAGCGATTGAAAATGGTCTGGCGTTTCAACGCC AGACCGTAGGGCGCGCCTACCTGTGACGG
downstream P22 *att*B	**LT2-stbB-cat-2c**	GGGACAGTCGCTGCCACCCTTCAATACGCCGTTTCGTATAA ATAATTGTCGGAATAGGAACTTCATTTAAATGGC
***cat* marker 29 kb**	**LT2-STM0352-cat-1m**	CGTTTCCCTCTTTACCGCAGCGTGTCGGCCATTCCGCAACG CTGCCGCAGGGCGCGCCTACCTGTGACGG
downstream P22 *att*B	**LT2-STM0352-cat-2c**	CCCGTCAGGAACGCTTGACCTTTCCTTCGTTGTAACGCCTA GCCTTTGCCGGAATAGGAACTTCATTTAAATGGC
P22 Δ*int* (same primers used for ES18 Δ*int)*	**P22-Aint-3m**	CCCGCTCGTTTTAATGCTGCCCTCCATGCAGTATTAGCGTC ATAGTGTGTAGGCTGGAGCTGCTTCG
	P22-Aint-4c	GAGGAGAAGGCGCATAAGAAGTCGCTGGATGATGACAAGA GTCGGGGTCCATATGAATATCCTCCTTAG
P22 Δ*xis*	**P22-Axis-1m**	ATGTCATCACCCGCGCTCACCTGGACAGTATGCAGCGGAG ATTGAAGTGCTGTGTAGGCTGGAGCTGCTTCG

	P22-Axis-2c	GAAACAGCGGAGTAAACATGGAATCACACAGCCTCACACTT GATGAGGCCGGTCCATATGAATATCCTCCTTAG
P22 Δ*int-xis*	**P22-Aint-3m**	CCCGCTCGTTTTAATGCTGCCCTCCATGCAGTATTAGCGTC ATAGTGTGTAGGCTGGAGCTGCTTCG
	P22-Axis-2c	GAAACAGCGGAGTAAACATGGAATCACACAGCCTCACACTT GATGAGGCCGGTCCATATGAATATCCTCCTTAG
P22 Δ*orf12*	**P22-Aorf12-1m**	GGTGGCTTGCTGATTGGCGGATTAACACCAACCGCCAGTG ACGTTCTGGCTGTGTAGGCTGGAGCTGCTTCG
	P22-Aorf12-2c	GCGGCTTCCTGATGGATGACAGGCGCTTTACAAGCTCGTCC ATCGCTCTGGGTCCATATGAATATCCTCCTTAG
ES18 Δ*xis*	**ES18-Axis-1m**	TATGCCATCACCCGCGCTCACGGCGACAGTATGCATCGGA GACTGAAGAGTGTGTAGGCTGGAGCTGCTTCG
	ES18-Axis-2c	AGCAATAACAATCCTCGCACTCGCGGGGATTTCTTTTATCC GGAGTAACCGGTCCATATGAATATCCTCCTTAG
ES18 Δ*int-xis*	**P22-Aint-3m**	CCCGCTCGTTTTAATGCTGCCCTCCATGCAGTATTAGCGTC ATAGTGTGTAGGCTGGAGCTGCTTCG
	ES18-Axis-2c	AGCAATAACAATCCTCGCACTCGCGGGGATTTCTTTTATCC GGAGTAACCGGTCCATATGAATATCCTCCTTAG

Excision	**Primers**	**Sequence (5'-3')**
P22 and ES18	**LT2-attB-1m**	TCAGCACGCAGAAATTACATG
	LT2-attB-2c	ACGGAATCATTAACATGATGG

Circularisation	**Primers**	**Sequence (5'-3')**
P22	**P22-gtrA-4m**	GTTTTGATCGATACAAGCGATC
	P22-int-7c	CCTGACTGAACATGCTCGAC
ES18	**ES18-orf33-1m**	TCATCAACAGTGCGACAGATG
	P22-int-7c	CCTGACTGAACATGCTCGAC

Housekeeping	**Primers**	**Sequence (5'-3')**
P22 and ES18	**LT2-rapA-1m**	CGCTGAGCTGGCGGTTGAAGG
	LT2-rapA-2c	TGCCGCGAGCAACATGAAGCG

Plasmid	Primers	Sequence (5'-3')	Comment
pBAD18			
pJP2534	**P22-int-5mS**	ACGCGTCGACCAAATACTTACGTATTATTCGTGCC	
	P22-xis-4cE	CCGGAATTCATTCTACGACATCGCTAACGC	
pBT2bgal			
pJP2563	**pp1-HF-1_FB**	AGTAGGATCCCAAAAGTAGGGGCCTTTTCTGCACCTTTTACAGC	*celA* left flank
	pp1-tetM_HF-1_R	CTCAAATTGCGAGATTTGGGTTGCCTTTGTTTCTTGATCAAATTTGAACAAAGGACTGAAACGATATGTCAATTTTGAAAAATG	
	tetM-F	GATCAAGAAACAAAGGCAACCCAAATCTCGCAATTTGAG	*tetM* cassette
	tetM-RS	GTCAGTCGACGATCTTGTATCATTACTCCATGTATCTATTGATG	
	pp1-HF-1_FB	AGTAGGATCCCAAAAGTAGGGGCCTTTTCTGCACCTTTTACAGC	*celA* left flank-*tetM* fusion
	tetM-RS	GTCAGTCGACGATCTTGTATCATTACTCCATGTATCTATTGATG	
	pp1-HF-1_FS	CATTTTTCAAAATTGACATATCGTTTCAGTCCTTTGTTCAAATTTGTCGACTTATACGTTTTGTGCTTCGACTTTTTCAAGGGTTTCTTC	*celA* right flank
	pp1-HF-1_RH	TGCAAAGCTTGTTTTCATTTTGTTTATGTCATCTGAAGTCAAAGATGCG	
pJP2564	**pp1-HF1_FB**	ATGAGGATCCGGCTACAACCAATTATTAGCTGACGATTATGTG	EF0370 left flank
	pp1-tetM_HF1-R	CTCAAATTGCGAGATTTGGGTTGCCTTTGTTTCTTGATCTTAAATTGCTGTTAAATTAGGGTTATTGATATTTTTAAAATAAAAATC	
	tetM-F	GATCAAGAAACAAAGGCAACCCAAATCTCGCAATTTGAG	*tetM* cassette
	tetM-RS	GTCAGTCGACGATCTTGTATCATTACTCCATGTATCTATTGATG	
	pp1-HF1_FB	ATGAGGATCCGGCTACAACCAATTATTAGCTGACGATTATGTG	EF0370 left flank-*tetM* fusion
	tetM-RS	GTCAGTCGACGATCTTGTATCATTACTCCATGTATCTATTGATG	
	pp1-HF1_FX	AAATATCAATAACCCTAATTTAACAGCAATTTAATCTAGAATTAAACAATCACTAAACAGAGGCTTGTCGACAG	EF0370 right flank
	pp1-HF1_RNh	GTCTGCTAGCTGGCGCAAAATGTTCATTATCTTGTGAAAAAAC	
pJP2565	**pp1-HF2_FB**	TGCAGGATCCACAACAATGCTAGATGCAGTTTTAGATGCGGACTC	EF0370 left flank
	pp1-tetM_HF2-R	CTCAAATTGCGAGATTTGGGTTGCCTTTGTTTCTTGATCTTAGGCTGAGTGTCCTACGATTGTGCTACCTGAGTAACC	
	tetM-F	GATCAAGAAACAAAGGCAACCCAAATCTCGCAATTTGAG	*tetM* cassette
	tetM-RS	GTCAGTCGACGATCTTGTATCATTACTCCATGTATCTATTGATG	
	pp1-HF2_FB	TGCAGGATCCACAACAATGCTAGATGCAGTTTTAGATGCGGACTC	EF0370 left flank-*tetM* fusion
	tetM-RS	GTCAGTCGACGATCTTGTATCATTACTCCATGTATCTATTGATG	

	pp1-HF2_FS	CTCAGGTAGCACAATCGTAGGACACTCAGCCTAAGTCGACTCAGCAATGATAAAAATAGACGTAAGCAGCTTC	EF0370 right flank
	pp1-HF2_RNh	ATGTGCTAGCATCATCAAATTGCAAATAACGGCAACCTCTCTC	
pJP2566	**pp1-HF4_FB**	ATGTGGATCCCATTTTAGTTAAATCAGTAATTTTCAATATCGAGGG	EF0480 left flank
	pp1-HF4_RS	GAAGCTATCTTAAAATTTAAAATTAACTAAGTCGACCTAAAAAAAGCCCCTTTCCACTTTTTCTG	
	tetM-FS	GTACGTCGACGATCAAGAAACAAAGGCAACCCAAATCTCGCAATTTGAG	*tetM* cassette
	tetM-RS	GTCAGTCGACGATCTTGTATCATTACTCCATGTATCTATTGATG	
	pp1-HF4_FS	CAGAAAAAGTGGAAAGGGGCTTTTTTTAGGTCGACTTAGTTAATTTTAAATTTTAAGATAGCTTC	EF0480 right flank
	pp1-HF4_RH	GTCTAAGCTTCTGATTAAAACTTTATTATGATGTCATAGTGAAG	
pJP2568	**2196839_FB**	ATGTGGATCCGAAATAAGCAATAATCATTGGTCAACGCCTCTC	EF2276 left flank
	2196839_RS	TAGATTATCGAATATTTTCGAAATTTAATTGAATGACAAGTCGACTCAAAGTACGAGGCGATACATTGCTGATAATTTTTTAGAGTATTG	
	tetM-F	GATCAAGAAACAAAGGCAACCCAAATCTCGCAATTTGAG	*tetM* cassette
	tetM-RS	GTCAGTCGACGATCTTGTATCATTACTCCATGTATCTATTGATG	
	2196839_FB	ATGTGGATCCGAAATAAGCAATAATCATTGGTCAACGCCTCTC	EF2276 left flank-*tetM* fusion
	tetM-RS	GTCAGTCGACGATCTTGTATCATTACTCCATGTATCTATTGATG	
	2196839_FSa	TGCAGTCGACTTGTCATTCAATTAAATTTCGAAAATATTCGATAATCTA	EF2276 right flank
	2196839_RNh	GTCAGCTAGCTCAGGTGTAGAAAATTTTACTGTAGATTTTGCC	

Table S4. Plasmids used in this study.

Plasmid	Description	Reference
pKD46	Amp^R. Thermosensitive plasmid with Red lambda system	[3]
pCP20	Amp^R. Thermosensitive plasmid with FLP recombinase	[3]
pBAD18	Amp^R. Expression vector	[4]
pBT2βgal	Amp^R, GN; Cm^R, GP. Vector for allelic replacement	[5]
pRN6680	pBluescriptΩ2.9-kb pMVN6 *Sma*I-*Hind*II [*tetA(M)*]; Amp^R, Tet^R	[6]
pJP2534	pBAD18 *int-xis* P22	This work
pJP2563	pBT2βgal with *tetM* from pRN6680 positioned between *E. faecalis* V583 flanking sequences at *celA*; Cm^R, Tet^R	This work
pJP2564	pBT2βgal with *tetM* from pRN6680 positioned between *E. faecalis* V583 flanking sequences at *EF0370*; Cm^R, Tet^R	This work
pJP2565	pBT2βgal with *tetM* from pRN6680 positioned between *E. faecalis* V583 flanking sequences at *EF0394*; Cm^R, Tet^R	This work
pJP2566	pBT2βgal with *tetM* from pRN6680 positioned between *E. faecalis* V583 flanking sequences at *EF0480*; Cm^R, Tet^R	This work
pJP2568	pBT2βgal with *tetM* from pRN6680 positioned between *E. faecalis* V583 flanking sequences at *EF2276*; Cm^R, Tet^R	This work

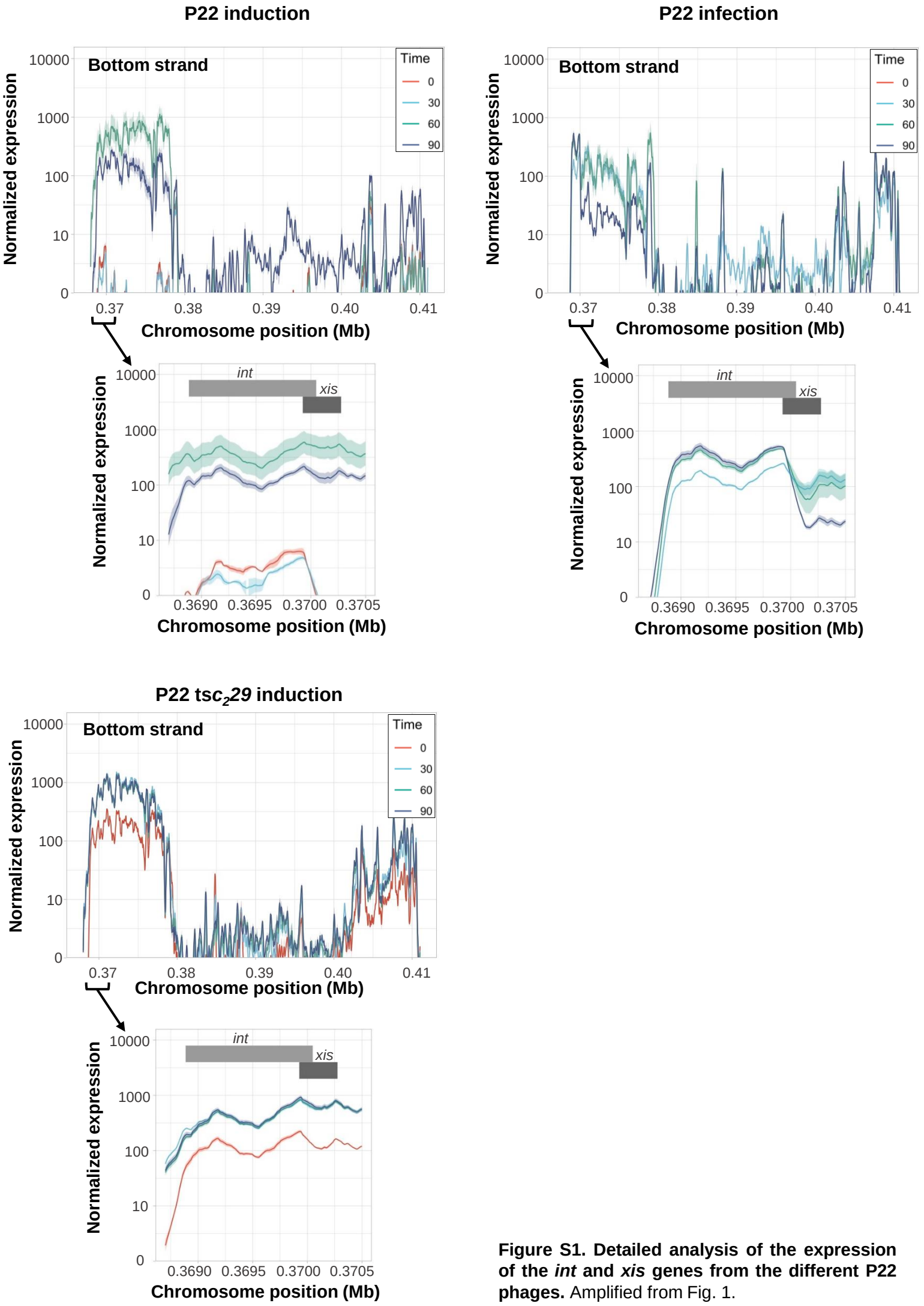
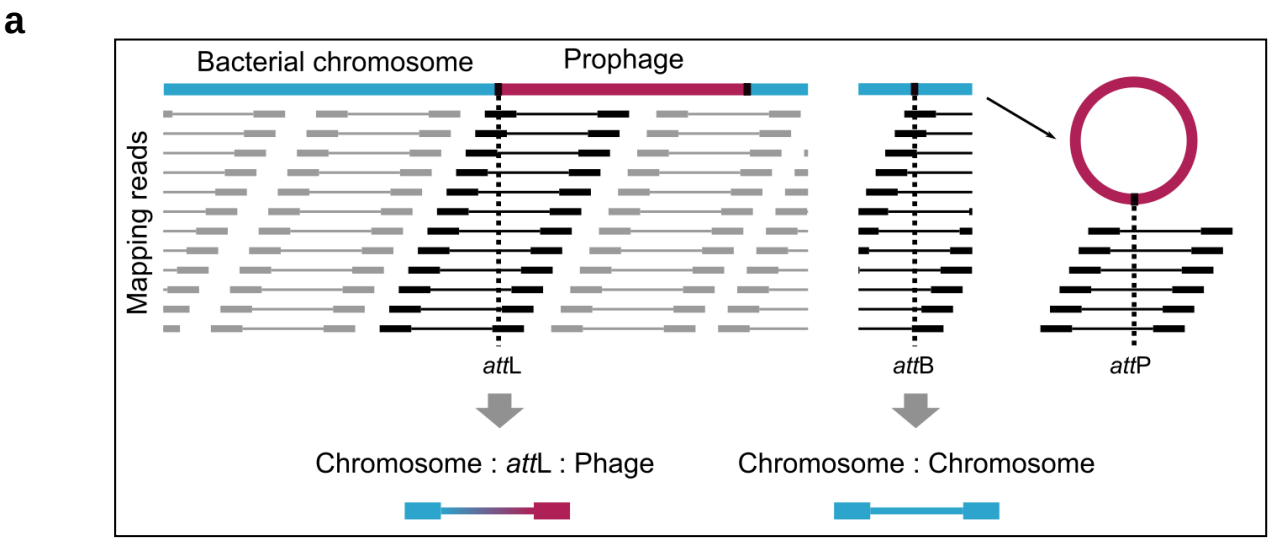
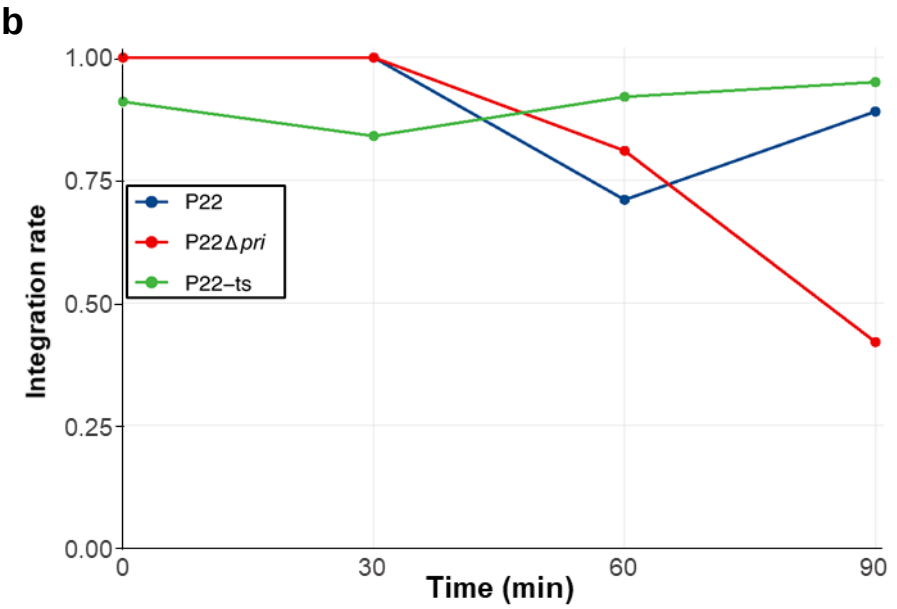


Figure S1. Detailed analysis of the expression of the *int* and *xis* genes from the different P22 phages. Amplified from Fig. 1.





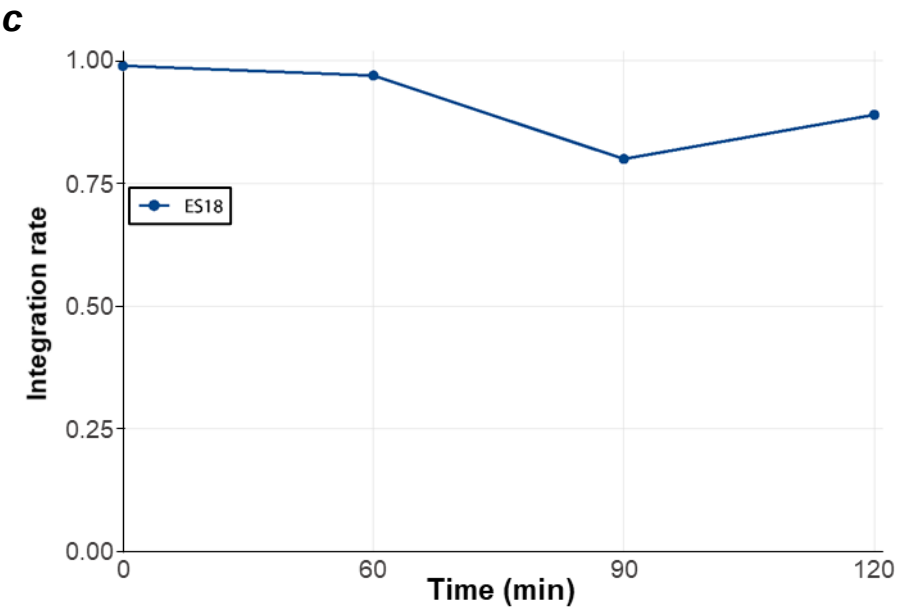


Figure S2. Prophages excise late from the bacterial chromosome.

Schematic representation of sequencing reads used for calculation of integration rate **(a)**, *att*L (left site attachment site) and *att*B (bacterial attachment site). To gain insight into the timing of phage excision, the proportion of integrated phage at each timepoint was calculated by dividing the number of chromosome-*att*L reads by the total number of *att*L/*att*B spanning reads (sum of chromosome-*att*L phage region reads plus reads spanning the *att*B region), obtained from the same sample, for phages P22, P22 Δ*pri* and P22 tsc_2*29* **(b),** and for ES18 **(c)**.

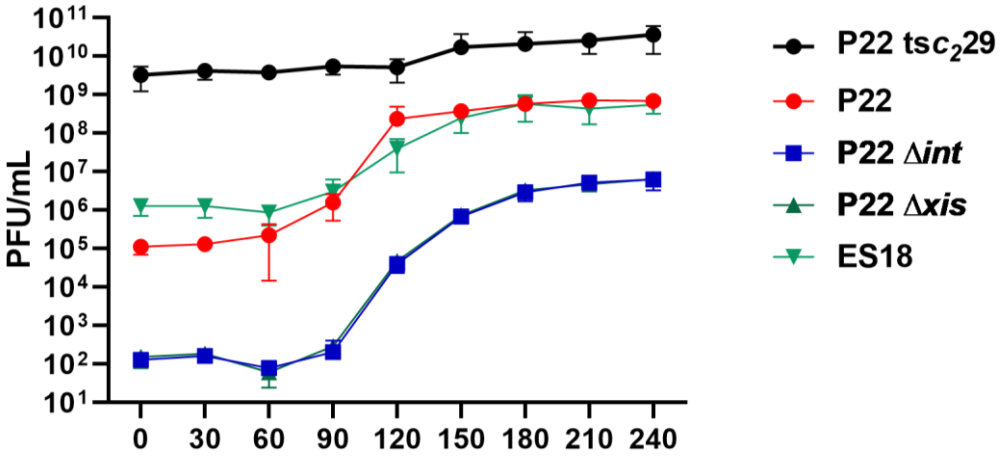


Figure S3. Phage production over a time course experiment after induction.

The lysogenic strains for phage P22, P22 Δ*int*, P22 Δ*xis,* P22 tsc_2*29* and ES18, were mitomycin C or thermal-induced to activate the prophages, samples were taken before (0) and at different time points (every 30 minutes) after induction and the phage titters evaluated (plaque forming units per milliltre: PFU/mL). The means and standard deviations from three independent experiments are presented (*n*=3).

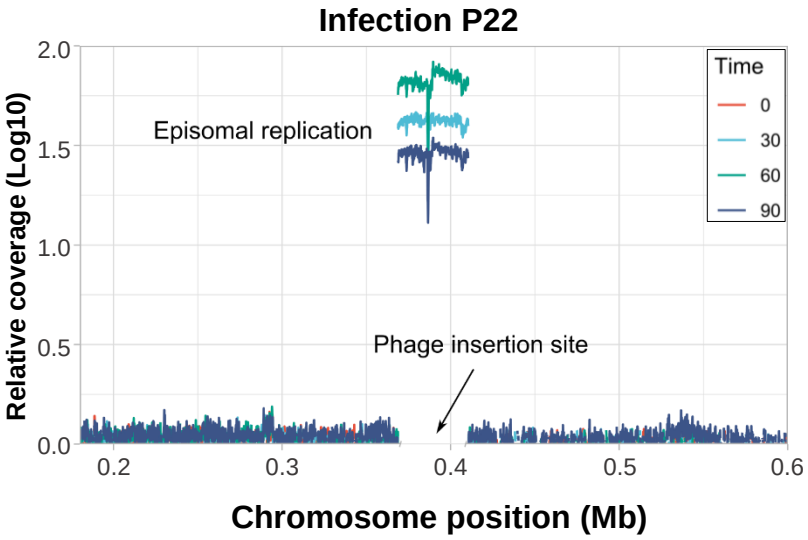


Figure S4. Phage P22 replicates extra-chromosomally after infection.

The relative abundance of phage genomic DNA and the chromosomal regions proximal to where P22 integrates is represented. Samples were analyzed at 0 (red), 30 (cyan), 60 (green), and 90 min (blue) after phage P22 infection. Relative coverage is the DNA relative to the average bacterial genomic coverage (excluding phage).

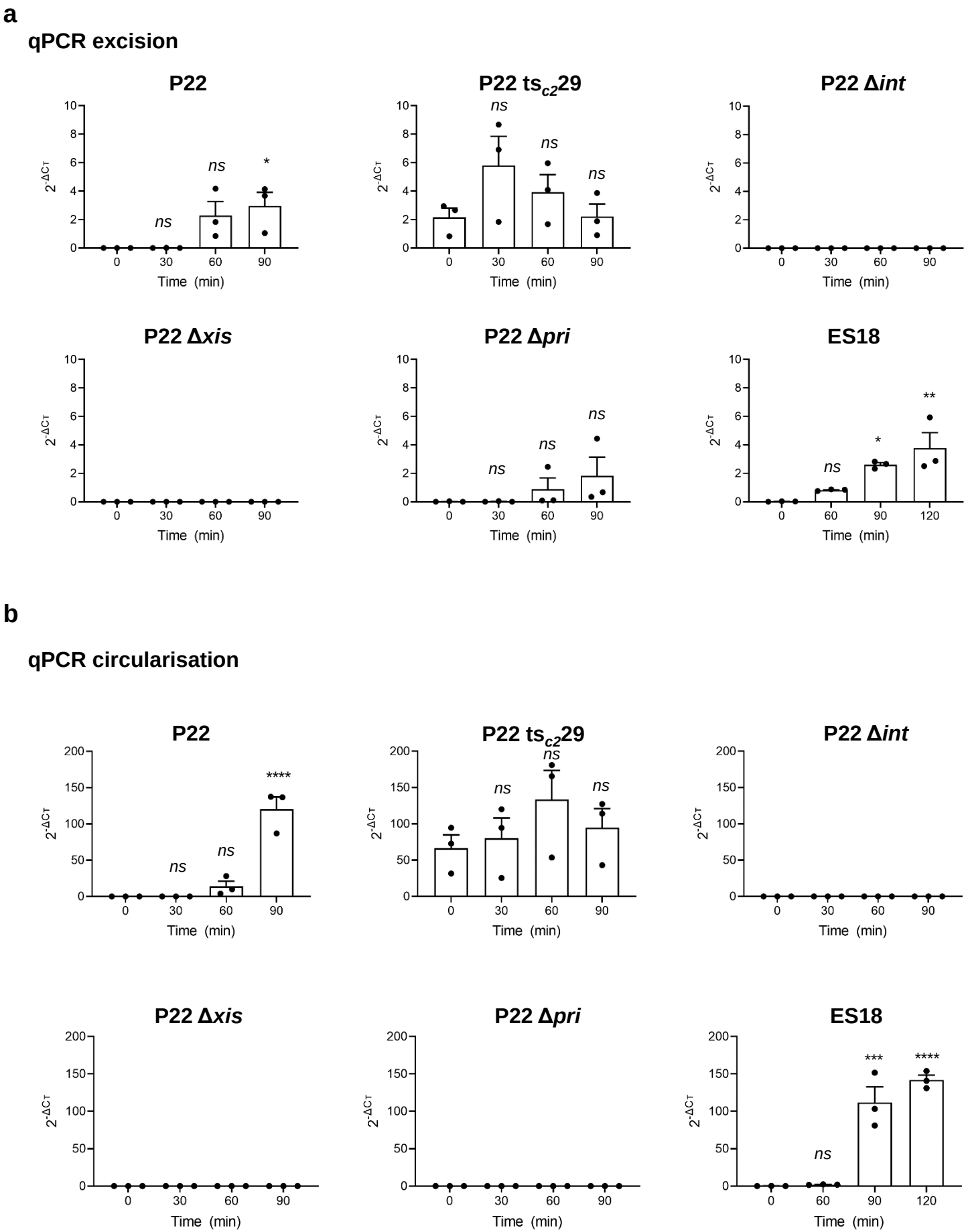


Figure S5. qPCR verification of the strains used in WGS

Analysis of the excision **(a)** or circularisation **(b)** of the different prophages after MC induction, using qPCR. Samples were analysed at 0, 30, 60, and 90 min after induction of the wt or mutants P22 prophages, or at 0, 60, 90, and 120 min after induction of the ES18 prophage (using mitomycin C or temperature-shift, as appropriate). Relative gene expression levels were analyzed by using the $2^{-\Delta CT}$ (where *CT* is threshold cycle). Error bars indicate standard deviation from the mean of three independent experiments. For all panels, values are means (n = 3 independent samples). A one-way ANOVA with Dunnett's multiple comparisons test was performed to compare time 0 against the other timepoints. Adjusted p values were as follows: *ns*>0.05; *p≤0.05; **p≤0.01; ***p≤0.001; ****p≤0.0001. The exact statistical values for each of the conditions tested are listed in Table S1.

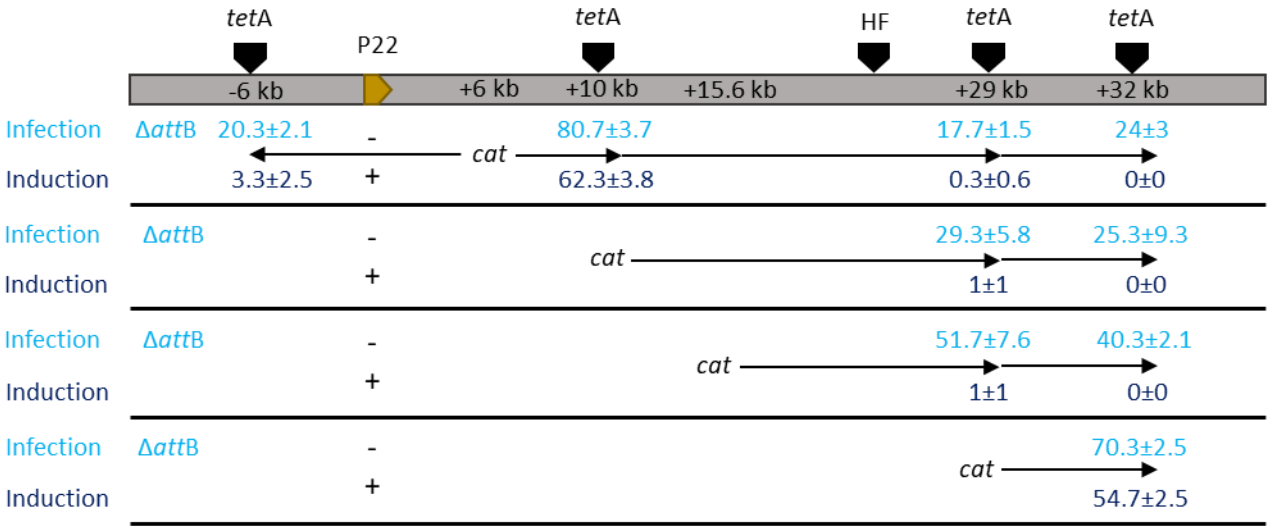


Figure S6. Lateral transduction occurs by the headful mechanism.

Co-transduction frequencies for strains containing both chloramphenicol (*cat)* and tetracycline (*tet*A) markers at varying distances apart. Lysates were tested for co-transduction of both markers by initially selecting for the *tet*A marker, with transductants subsequently scored for the *cat* marker. At least one hundred *tet*A transductants were tested from infection (cyan) or induction (blue) and the results are represented as a percentage (*cat*/*tet*A) x 100%. The *att*B for P22 is highlighted in orange and the headful limit (HF) is indicated. The + indicates a P22 lysogen (induction, LT), while the - indicates a non-lysogenic strain (infection, GT). The means and standard deviations from three independent experiments are presented (*n*=3). Our results indicate that for lysates generated by P22 infection, all co-transduction frequencies were inversely proportional to their distance apart, even if they were located in two different headfuls, indicating that DNA packaging initiated at random sites. However, when we tested lysates generated by SOS induction, co-transduction was only observed for markers within a headful, indicating that packaging had primarily initiated from the bona fide *pac* site, and confirming that P22-mediated LT uses the headful mechanism for packaging.

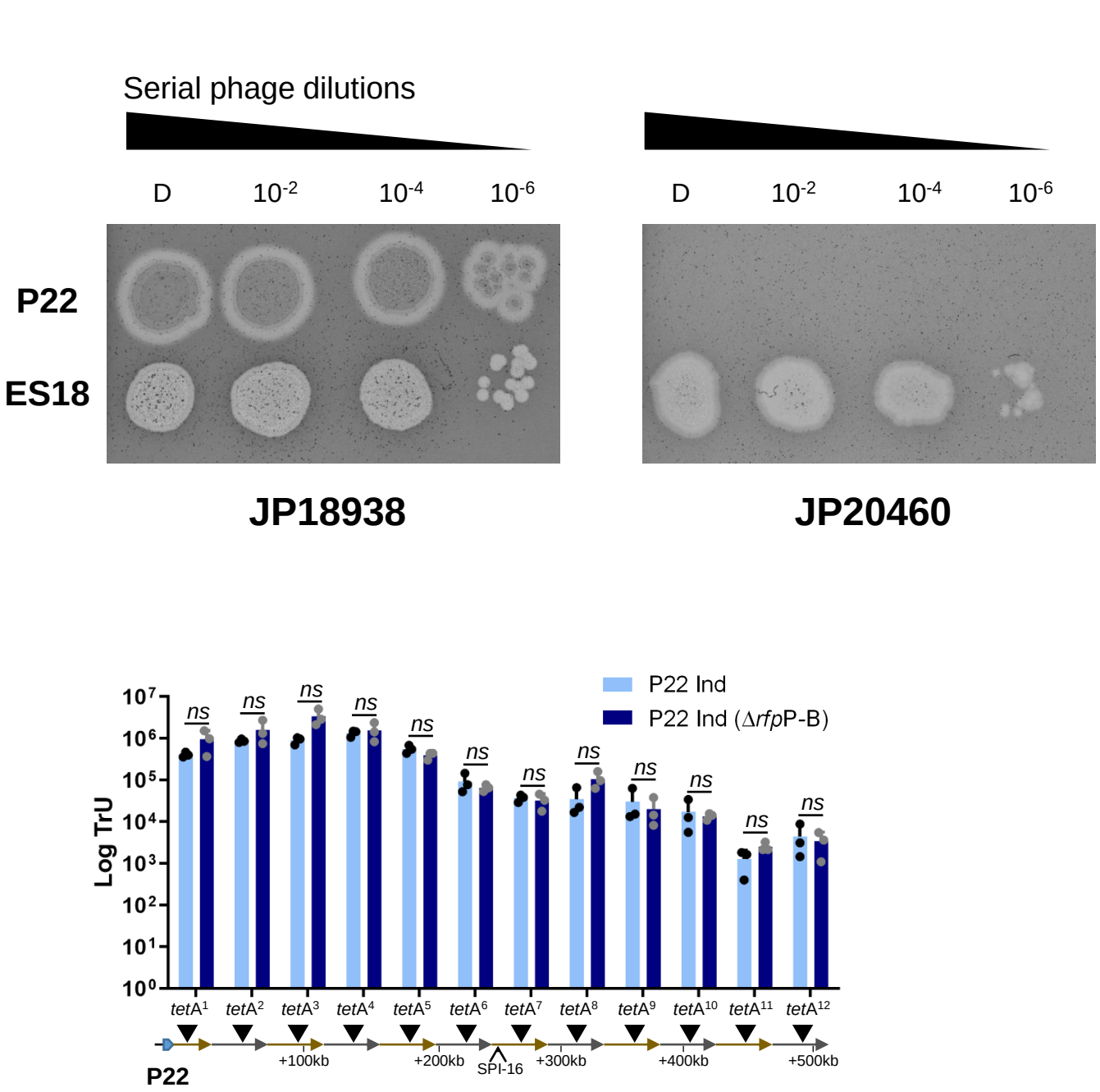


Figure S7. Phage P22 engages in lateral transduction in an infection-resistant host.

To rule out the possibility that P22 phages released early after SOS induction could superinfect the remaining cells to initiate packaging from the resident prophage genome, we deleted the *rfp*P-B genes that encode the P22 receptor and tested this strain for LT. **a,** Deletion of *rfp*PB renders LT2 insensitive to P22 infection. Serial dilutions of phages P22 and ES18 lysates were spotted onto lawns of non-lysogenic LT2 and non-lysogenic LT2 *ΔrfpP-B* strains. The presence of plaques indicates successful phage infection and replication via the lytic cycle. **b,** The transfer of tetracycline (*tet*A) markers located downstream of the P22 *att*B site, in twelve successive capsid headfuls (*tet*A$^{"n"}$), was tested after P22 induction (Ind) in either wild type LT2 or LT2 lacking the phage P22 receptor (Δ*rfp*P-B). Transduction units (TrU) per milliliter were normalized by PFU per milliliter and represented as the log TrU of an average phage titre (1 x 10^9 PFU). Error bars indicate standard deviation from the mean of three independent experiments. For all panels, values are means (n = 3 independent samples). An unpaired t test two-sided was performed to compare mean differences of infection and induction in each marker. Adjusted p values were as follows: *ns*>0.05; *p≤0.05; **p≤0.01; ***p≤0.001; ****p≤0.0001. The exact statistical values for each of the conditions tested are listed in Table S1.

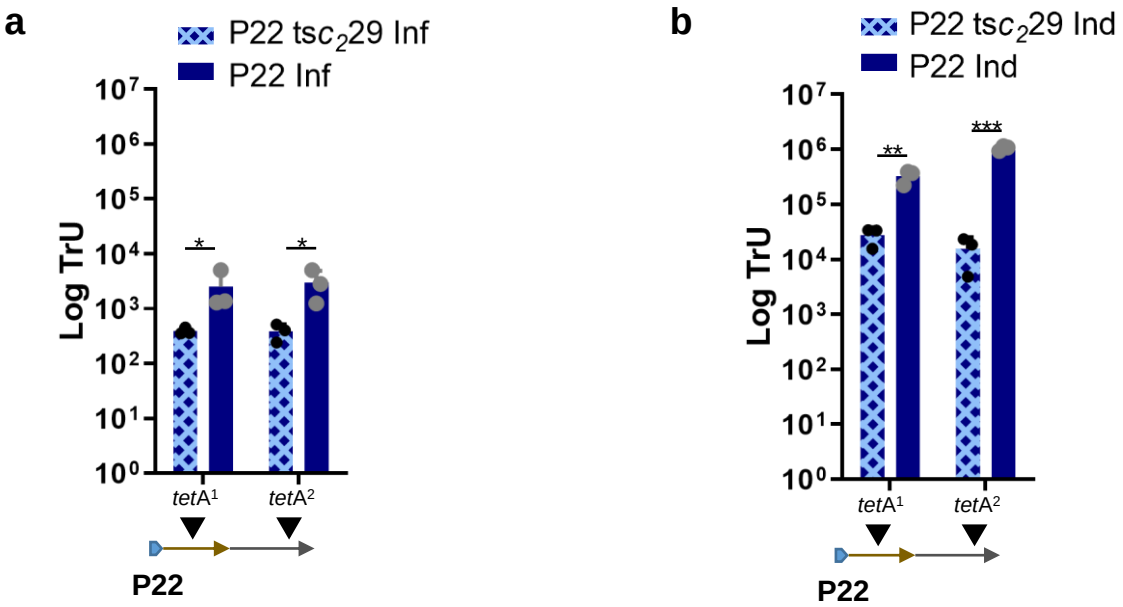


Figure S8. P22 ts$c_2$29 early excision produces low levels of lateral transduction.

a, The transfer by GT of the two first tetracycline (*tet*A) markers located downstream of the P22 *att*B site was compared for both P22 and P22 tsc_2*29* after phage infection (Inf). **b,** The transfer of the two first *tet*A markers located downstream of the P22 *att*B site was compared for both the P22 and the P22 tsc_2*29* prophages following induction (Ind). Transduction units (TrU) per milliliter were normalized by PFU per milliliter and represented as the log TrU of an average phage titre (1 x 10^9 PFU). Error bars indicate standard deviation from the mean of three independent experiments. For all panels, values are means (n = 3 independent samples). An unpaired t test two-sided was performed to compare mean differences of infection and induction in each marker. Adjusted p values were as follows: *ns*>0.05; *p≤0.05; **p≤0.01; ***p≤0.001; ****p≤0.0001. The exact statistical values for each of the conditions tested are listed on Table S1.

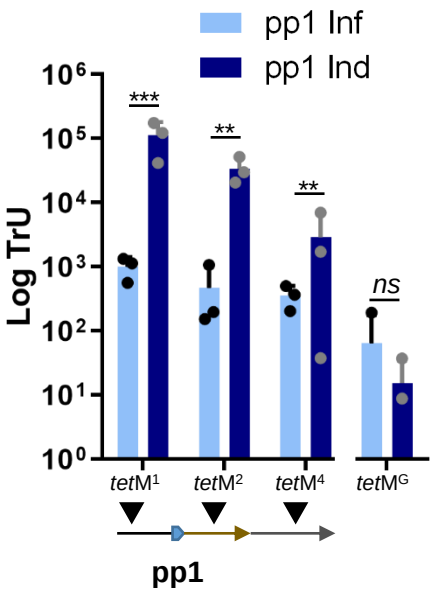


Figure S9. Enterococcal phage pp1 engages in lateral transduction.

The ability of phage pp1 to mobilise tetracycline (*tet*M) markers located downstream of the pp1 *att*B site in successive capsid headfuls (*tet*M$^{"n"}$), or from non-phage associated regions of the chromosome (*tet*M^{G}), was tested following induction (Ind) and infection (Inf). Transduction units (TrU) per milliliter were normalized by PFU per milliliter and represented as the log TrU of an average phage titre (1 x 10^9 PFU). Error bars indicate standard deviation from the mean of three independent experiments. Values are means (n = 3 independent samples). An unpaired t test two-sided was performed to compare mean differences of infection and induction in each marker. Adjusted p values were as follows: *ns*>0.05; *p≤0.05; **p≤0.01; ***p≤0.001; ****p≤0.0001. The exact statistical values for each of the conditions tested are listed in Table S1.

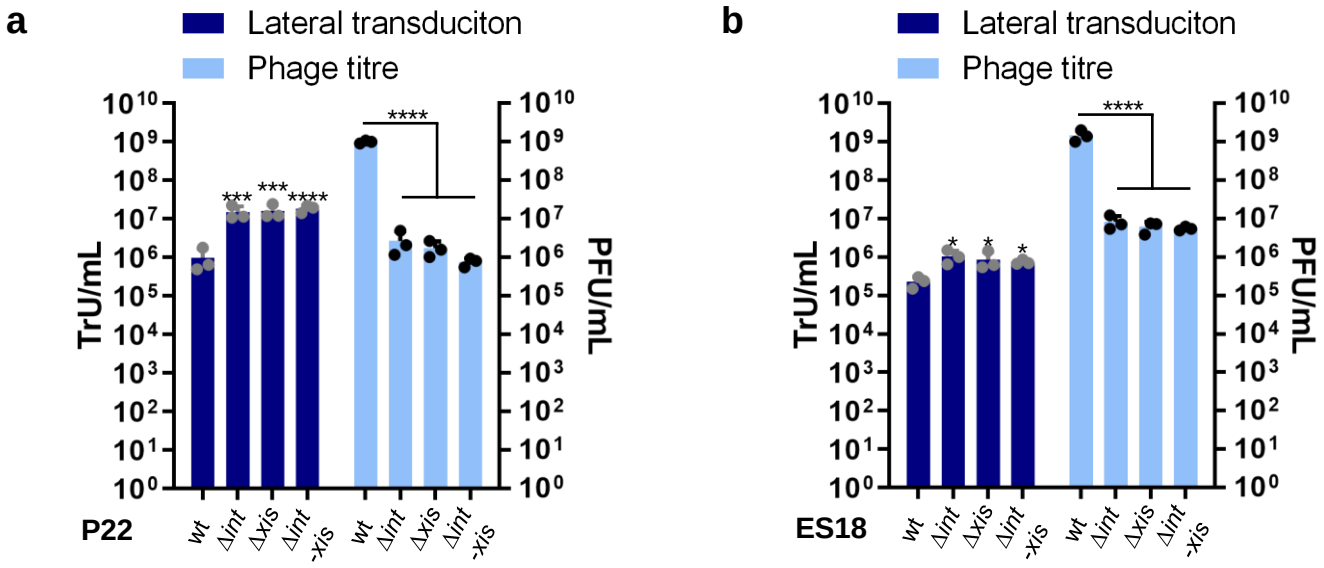


Figure S10. Effect of phage mutations on lateral transduction and phage formation.

a, P22 or P22-derivative (Δ*int*, Δ*xis* or Δ*int-xis*) prophages were mitomycin C induced and tested for their ability to transduce a *tet*A marker (TrU/ml) and produce viable phage particles (PFU/ml). **b,** The same experiment was performed using phage ES18 or ES18-derivates (Δ*int*, Δ*xis* or Δ*int-xis*). The means and standard deviations from three independent experiments are presented (*n*=3). A one-way ANOVA with Dunnett's multiple comparisons test was performed to compare mean differences between time WT and derivatives phages. Adjusted p values were as follows: *ns*>0.05; *p≤0.05; **p≤0.01; ***p≤0.001; ****p≤0.0001. The exact statistical values for each of the conditions tested are listed in Table S1.

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