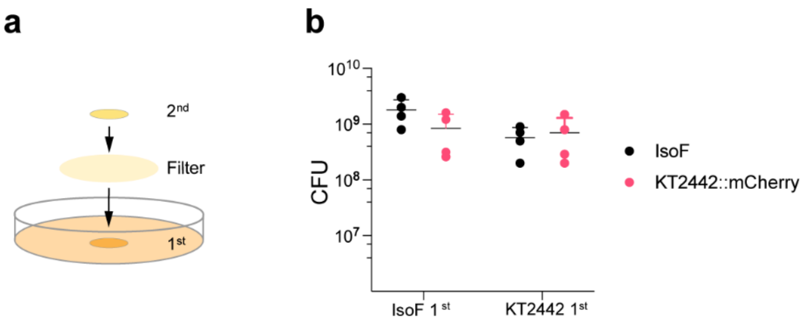
Entended Data

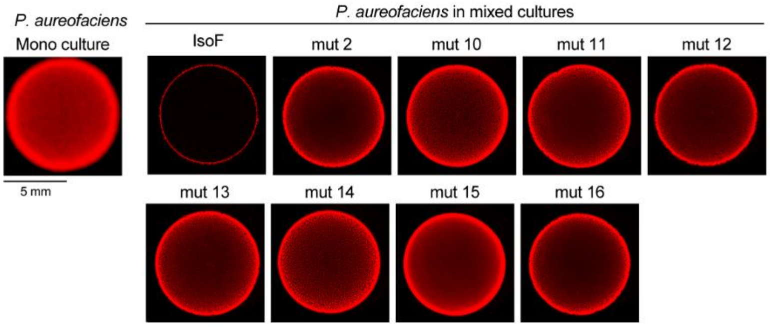
Extended Data Video

Extended Data Video 1. CDC of IsoF::Gfp with KT2442. KT2442 cells (grey, untagged) were killed by IsoF (green cells) in a contact-dependent manner. Dead cells (magenta) were visualized by staining with PI. The competition was followed for 4 hours by CLSM. Images were taken every 15 min.

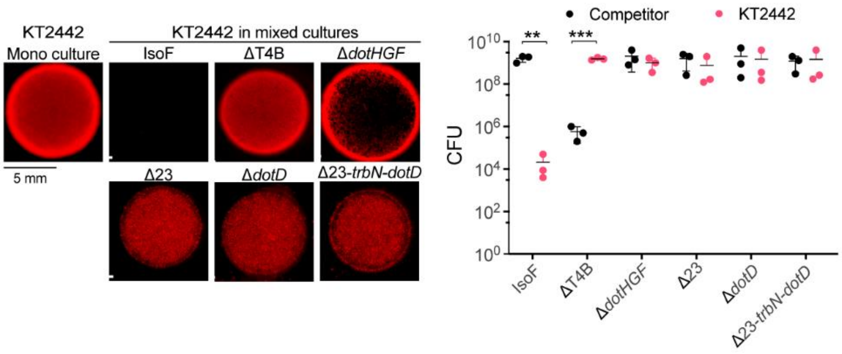
Extended Data Figures



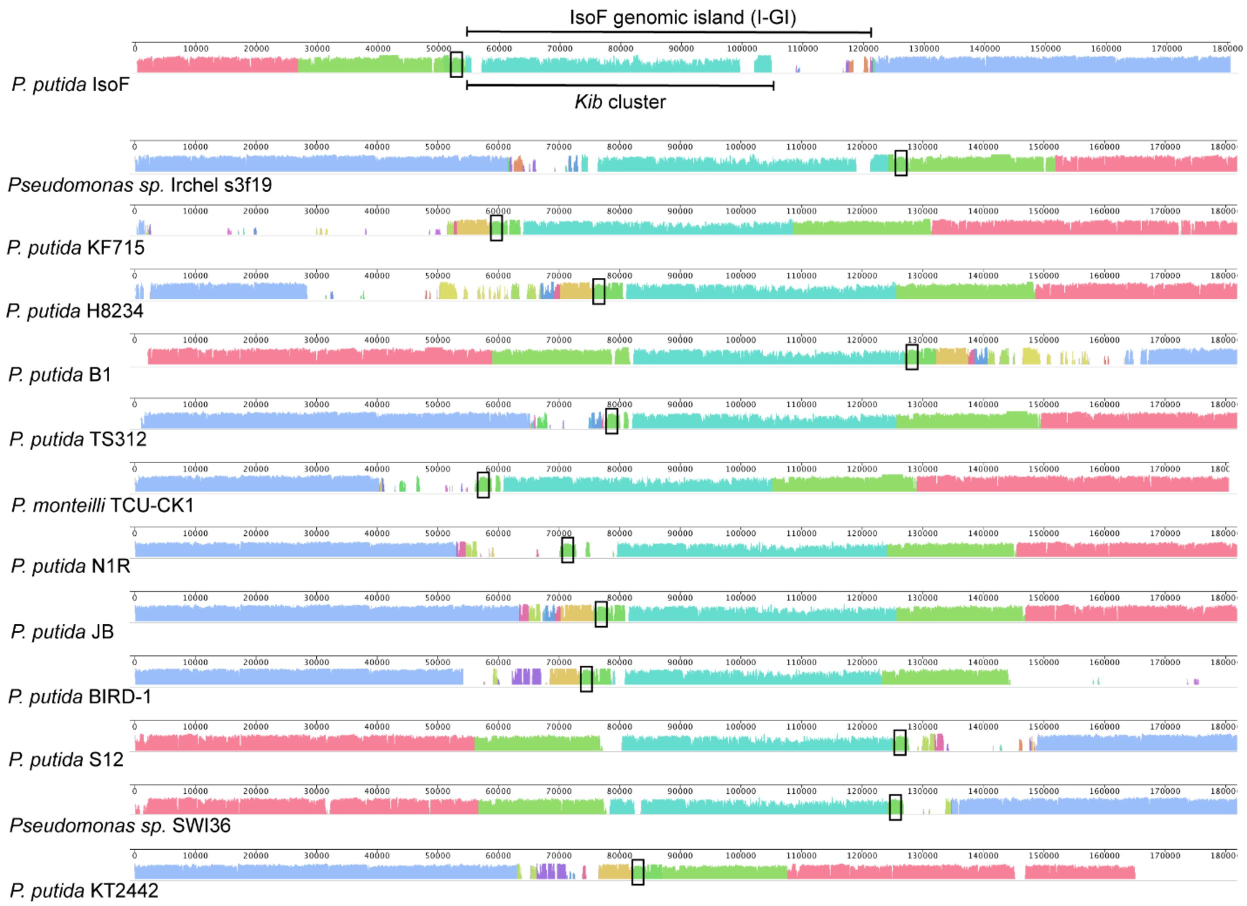
Extended Data Figure 1. Inoculation of KT2442::mCherry and IsoF separated by a filter. a, IsoF was inoculated on an ABC plate, a filter was placed atop the bacteria, which was subsequently inoculated with KT2442 or *vice versa*. **b**, CFUs were determined after 24 h of incubation. Data are mean ± s.d. from four independent replicates (n = 4).



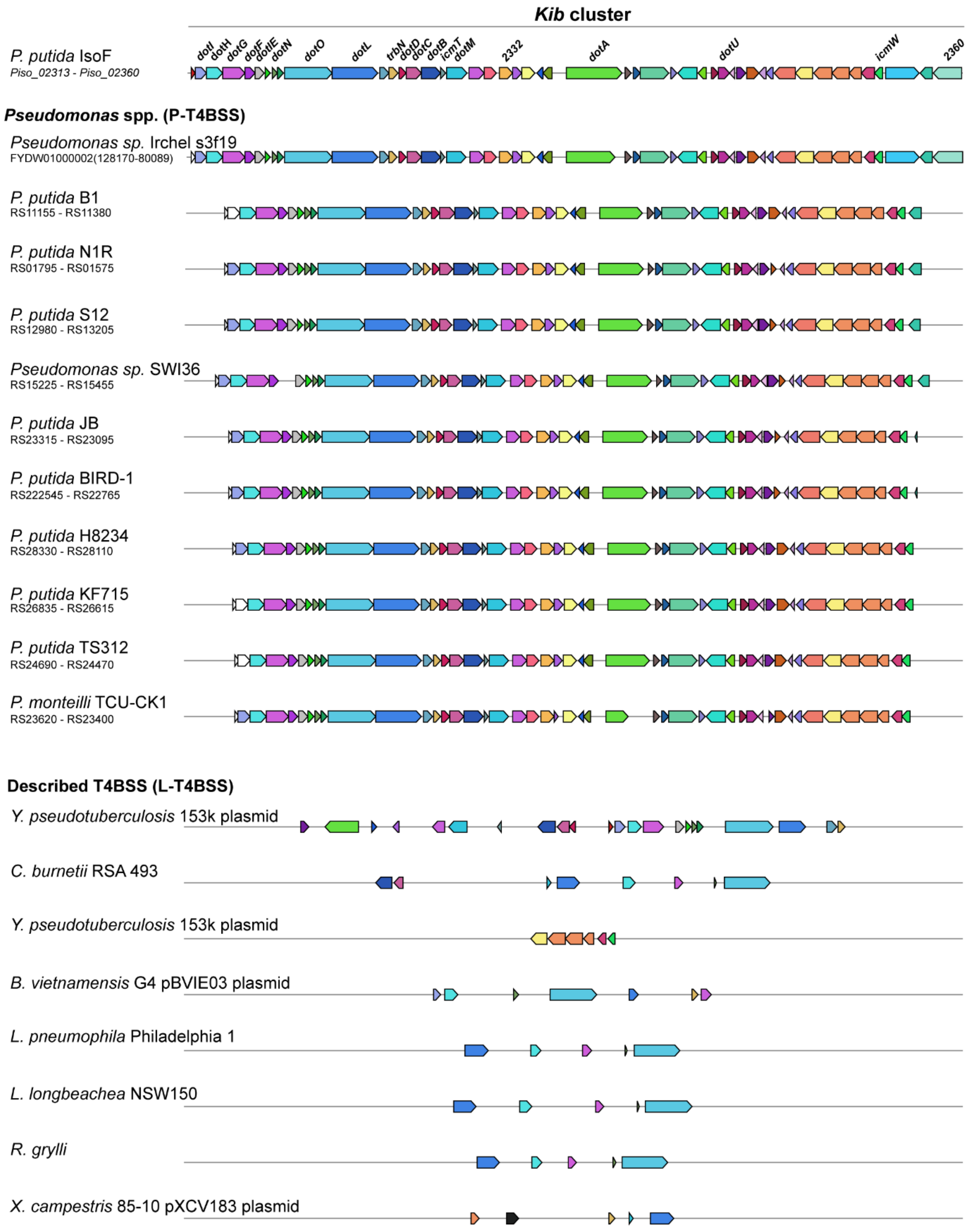
Extended Data Figure 2. Contact-dependent competition between the IsoF mini-Tn*5*-insertion mutants and *P. aureofaciens*. The mCherry (red) signal indicates the survival of *P. aureofaciens*::mCherry in competition with the mini-Tn*5* insertion mutants. Representative pictures of at least 3 replicates are shown.



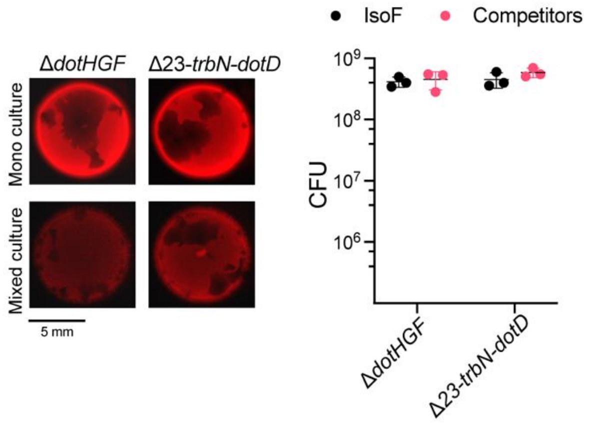
Extended Data Figure 3. Contact-dependent competition of defined mutants against KT2442. The wild-type strain and the deletion mutants Δ*dotHGF,* Δ23, Δ*dotD*, Δ23-*trbN-dotD* and ΔT4B were co-inoculated with KT2442 tagged with mCherry. Representative pictures of at least 3 replicates are shown. CFUs were determined after 24 h of incubation. Data are mean ± s.d. of three independent biological replicates (n=3). Unpaired t-test, ***P* < 0.01; ****P* < 0.001.



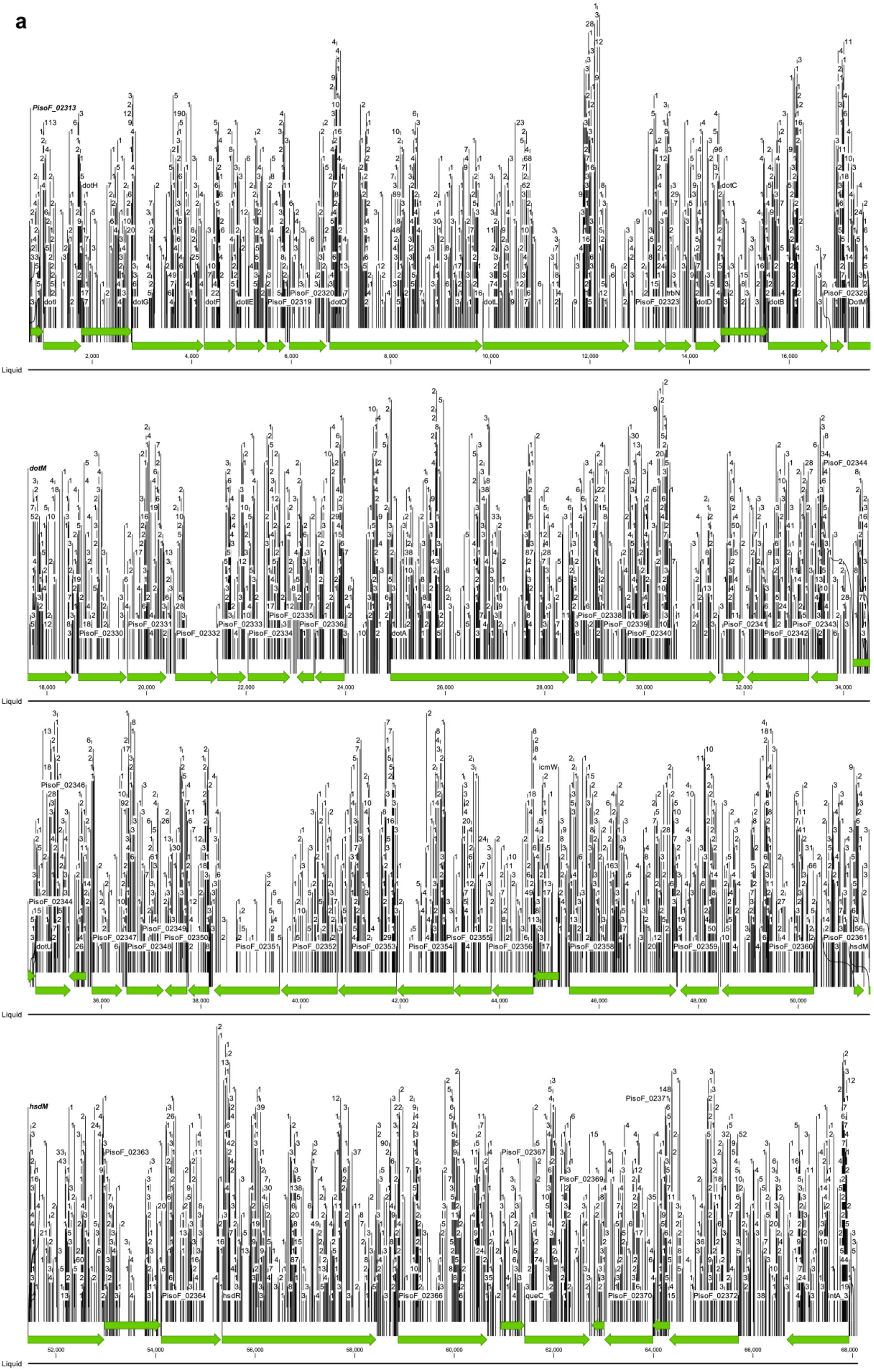
Extended Data Figure 4. Multiple genome alignment of *kib* loci. The *kib* regions from eleven *Pseudomonas* strains were compared and aligned with the IsoF-GI region using the Mauve software[1]. *P. putida* KT2440 (AE015451.2) was used as a reference strain not carrying this genomic element. Regions with identical colors represent local collinear blocks (LCB) of conserved DNA sequence. Low identity regions are shown without LCB. The *kib* gene cluster regions are indicated in light blue. The rectangular box (black) indicates the same position in a homologous region that is shared by all strains. The strains' accession numbers are: *Pseudomonas* sp. Irchel (NZ_FYDW01000002), *P. putida* KF715 (AP015029.1), *P. putida* H8234 (CP005976.1), *P. putida* B1 (NZ_CP022560.1), *P. putida* TS312 (NZ_AP022324.1), *P. monteilli* TC1-CK1 (NZ_CP040324.1), *P. putida* N1R (NZ_LT707061.1), *P. putida* JB (NZ_CP016212.1), *P. putida* BIRD-1 (CP002290.1), *P. putida* S12 (NZ_CP009974.1), *Pseudomonas* sp. SWI36 (NZ_CP026675.1).



Extended Data Figure 5. MultiGeneBlast alignment of IsoF *kib* genes with homologs of *Pseudomonas* and other T4BSS-encoding bacteria. MultiGeneBlast Alignment[2] of homologous regions from eleven *Pseudomonas* strains (P-T4BSS) and eight T4BSS-encoding bacteria as described by Nagai and Kubori, 2011[3] (L-T4BSS). Arrows represent genes and the color conservation indicates homology > 30 %. Gene identity threshold: 30%, hits mapped: 1000. Maximum distance between the genes in a locus is 10 kb.



Extended Data Figure 6. CDC between the IsoF wildtype and mutants Δ*dotHGF*::mCherry and Δ23-*trbN-dotD::*mCherry. Both mutant strains survived after 24 h of coincubation. CFUs are mean ± s.d. from three independent replicates (n=3). Representative images are shown.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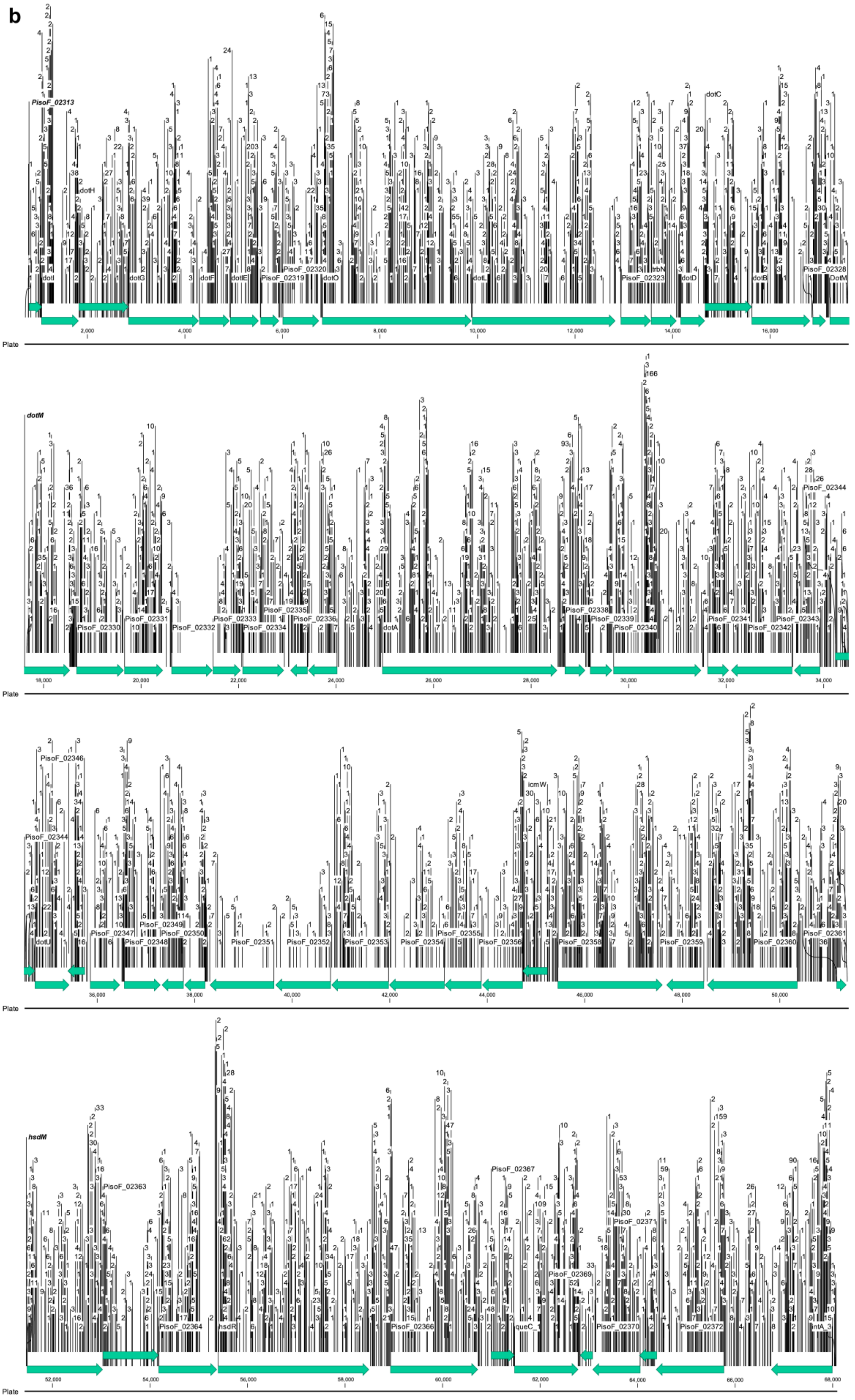
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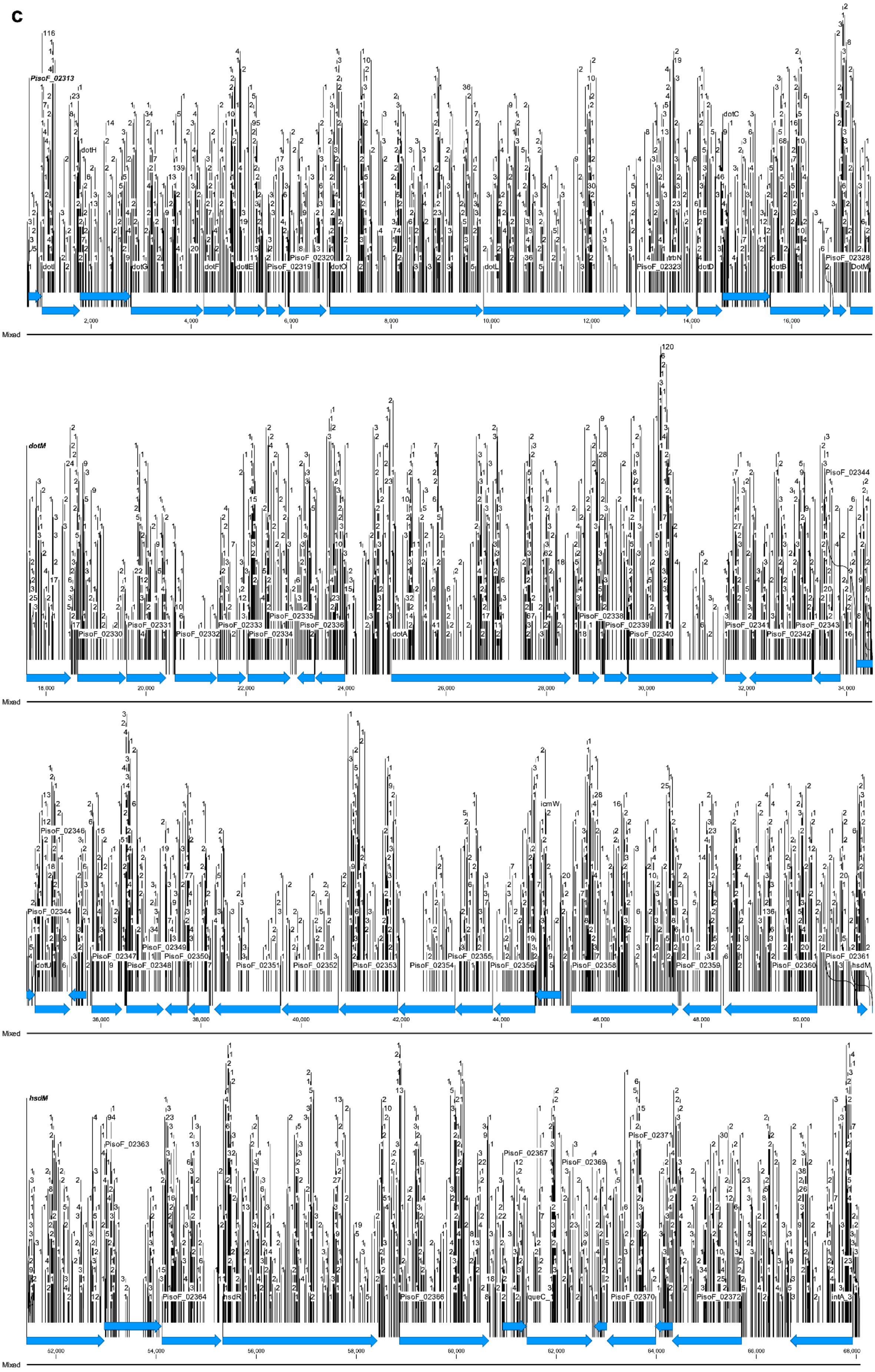c
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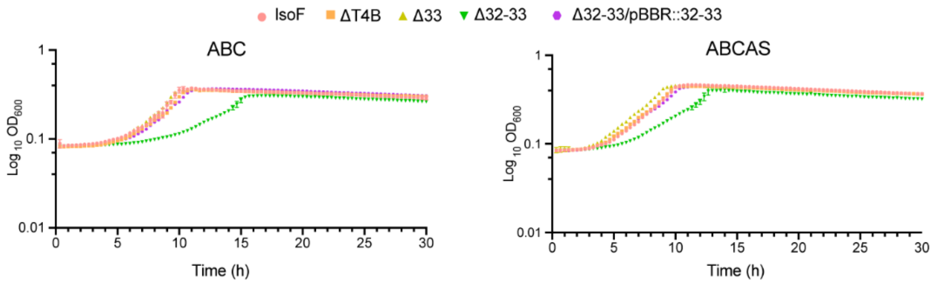
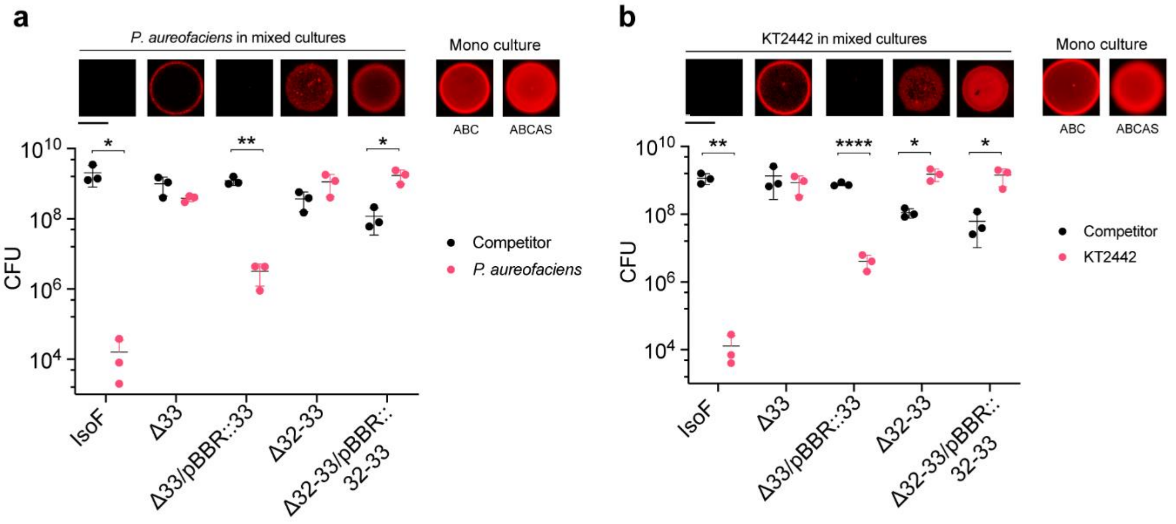
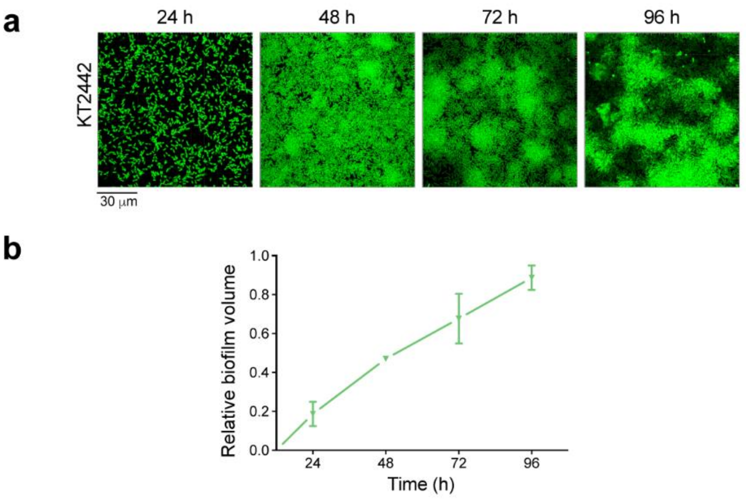
Extended Data Figure 7. Transposon insertions mapped on the genomic island of IsoF. The transposon insertion density in *Piso_02332* was greatly reduced compared to the rest of the GI. **a**, growth in liquid medium. **b**, Growth on an agar surface **c.** Growth on an agar surface in the presence of *P. aureofaciens*.



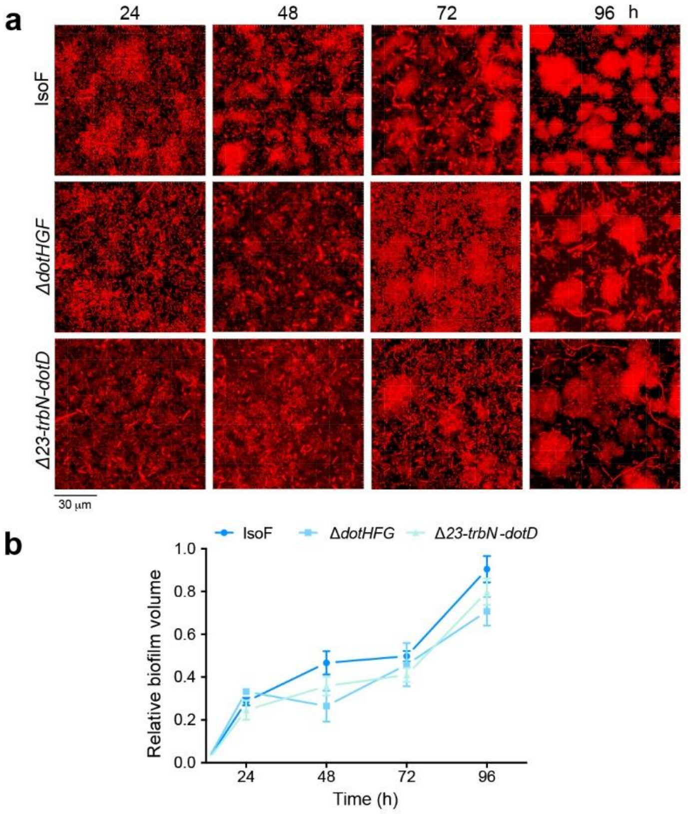
Extended Data Figure 8. Growth of IsoF and various deletion mutants in ABC minimal medium in the absence or presence of casamino acids (ABCAS). Data are mean ± s.d. of two biological replicates (n=2).



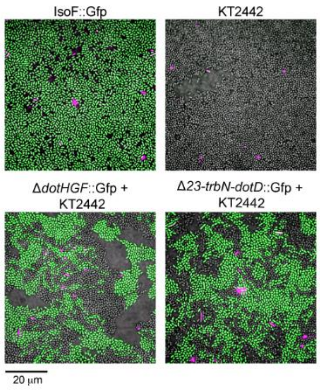
Extended Data Figure 9. CDC against a. *P. aureofaciens*::mCherry and b. KT2442::mCherry. CFUs were determined after 24 h of competition. Representative pictures of each competition are shown on top of the CFU values . CFUs are mean ± s.d. from three biological replicates (n=3). Unpaired t-test, **P* < 0.05; ***P* < 0.01; *****P* < 0.0001. Scale bar, 5 mm.



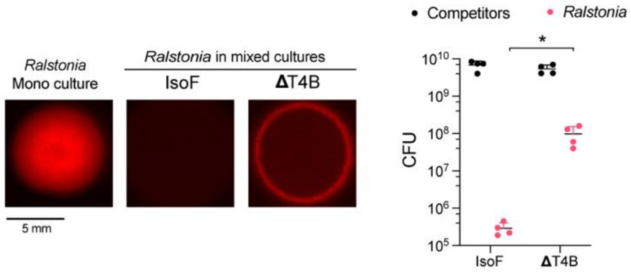
Extended Data Figure 10. **Biofilm development of KT2442::Gfp. a**, Representative pictures of biofilm development over 96 h. **b**, Quantification of biomass (relative volume) increase over time. Biofilms were visualized by CLSM using a 63 x 1.3 oil objective. Biomass (volume) of the biofilm was quantified by the Imaris software (Bitplane). Data are mean ± s.d. of three biological replicates (n=3).



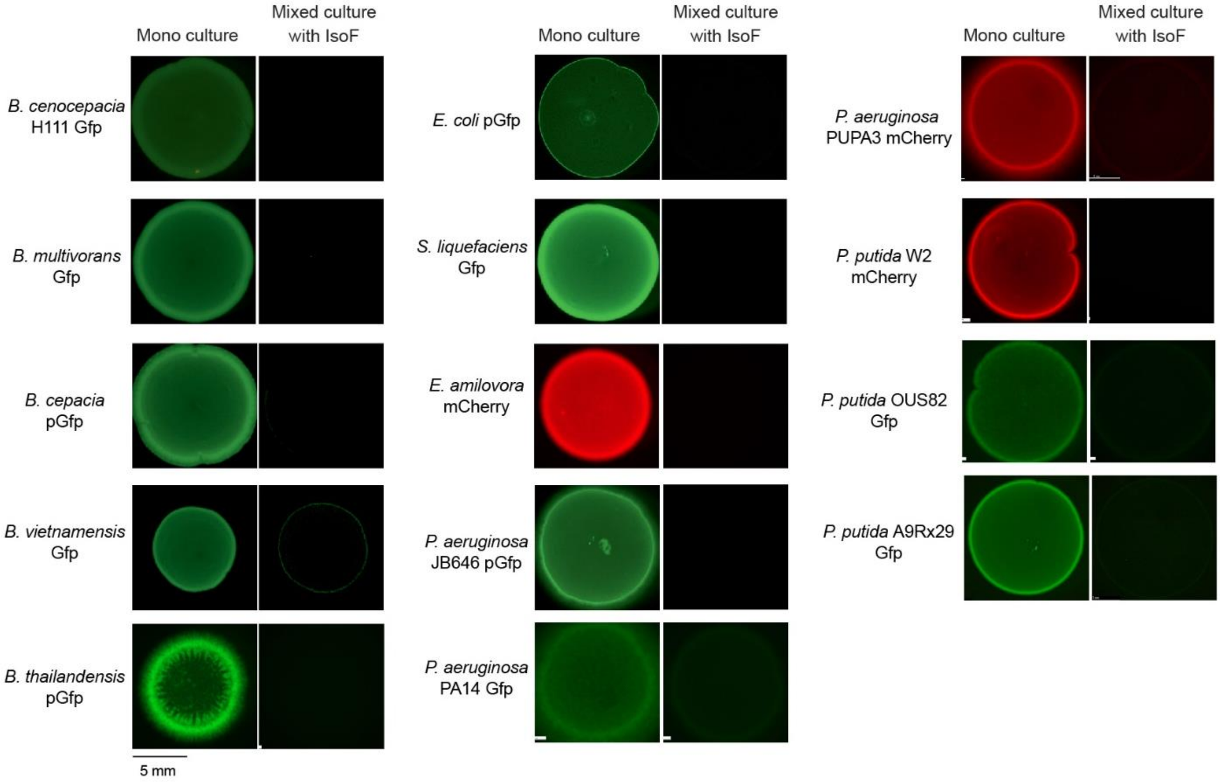
Extended Data Figure 11. **Biofilm formation of IsoF, Δ*dotHGF* and Δ23-*trbN-dotD*. a**, Representative LSCM images of biofilm development over 96 h. **b**, Quantification of biofilm biomass. Data are mean ± s.d of three independent replicates (n=3).



Extended Data Figure 12. CDC of Δ*dotHGF* and Δ23-*trbN-dotD* against KT2442. Representative images of the IsoF::Gfp and KT2442 monocultures and the competition experiments after 18 h of incubation are shown. The medium was supplemented with PI to visualize dead cells (magenta) are shown.



Extended Data Figure 13. CDC between IsoF and ΔT4B against *R. solanacearum* tagged with mCherry. *R. solanacearum* coexists with the ΔT4B mutant but is eradicated by the wild-type strain. CFUs were determined after 24h. Representative pictures shown. Data are mean ± s.d. of three independent experiments(n=3). Unpaired t-test, *$P < 0.05$.



Extended Data Figure 14. IsoF outcompetes a wide range of Gram-negative bacteria. Various fluorescently tagged bacteria were competed against the IsoF wildtype. Fluorescence images of the mono and mixed cultures after 24 h of incubation are shown. Lack of fluorescence indicates that IsoF outcompeted the bacterial strain.

Extended Data Tables

Extended Data Table 1. List of Pseudomonads carrying the T4BSS cluster elements and the comparison of the GC content, the nucleotide identity and the query cover shared with IsoF

Strain	GC content (%)			T4BSS gene cluster (%)		Isolated from
	T4BSS cluster	Genome [3]	Difference [4]	Query cover	Identity	
P. putida H8234	60.42	62.32	1.9	60	84.82	Clinical
P. putida BIRD-1	60.41	62.42	2.01	56	84.03	Rhizosphere
P. putida JB	60.36	62.51	2.15	55	84.05	Soil
P. putida N1R	60.36	62.65	2.29	57	84.05	Soil
P. putida S12	60.34	62.51	2.17	59	84.09	Soil
Pseudomonas sp. SWI36	60.36	62.54	2.18	57	85.02	Soil
P. putida KF715	60.28	62.65	2.37	61	84.81	Soil
P. putida B1	60.29	62.69	2.4	59	84.77	Soil
Pseudomonas sp. Irchel s3f19	60.24	62.76	2.52	75	99.67	Soil
P. monteilii TCU-CK1	59.67	62.64	2.97	64	84.8	Soil
P. putida TS312	59.8	62.92	3.12	61	84.8	Paper mill
P. putida IsoF – *kib* cluster [1]	60.09	63.34	3.25	-	-	Rhizosphere
P. putida IsoF – GI [2]	58.76	63.36	4.6	-	-	

[1] The GC percentage of the T4BSS cluster region

[2] The GC percentage of the entire IsoF genomic island (I-GI) including the T4BSS cluster, the Type I RM system and the integrase

[3] Genome GC content % excluding the correspondent T4BSS or T4BSS cluster/I-GI region

[4] Difference between the T4BSS cluster % minus the Genome %

Extended Data Table 2. Transposon sequencing results for each treatment

Treatments	Total no. of reads	No. of unique insertion sites	Insertion frequency (bp)	No. of essential genes
Liquid	7,364,199	764,392	7.7	607
Solid	7,054,399	649,830	9.1	616
Mixed	7,419,977	851,838	6.9	644

Extended Data Table 3. Unique transposon insertions found within the I-GI including *kib* cluster genes. Red highlighted row indicates the gene found to be essential in all treatments.

Locus_tag	Description	Liquid		Solid		Mixed	
		UID[1]	UIC[2]	UID	UIC	UID	UIC
PisoF_02313	hypothetical protein	0.080	21	0.071	19	0.080	21
PisoF_02314	type IV secretion protein DotI/IcmL	0.086	65	0.088	67	0.100	76
PisoF_02315	type IV secretion system protein IcmK/DotH	0.058	59	0.068	69	0.057	58
PisoF_02316	type IV secretion protein DotG/IcmE	0.094	136	0.080	115	0.101	146
PisoF_02317	type IV secretion protein DotF	0.120	75	0.113	70	0.097	60
PisoF_02318	type IV secretion protein DotIE	0.071	42	0.080	47	0.048	28
PisoF_02319	hypothetical protein	0.096	37	0.052	20	0.078	30
PisoF_02320	type IV secretion system protein DotN	0.075	56	0.072	54	0.067	50
PisoF_02321	type IV secretion system protein IcmB/DotO	0.095	292	0.089	273	0.086	263
PisoF_02322	type IV secretion system protein IcmO/DotL	0.064	188	0.060	176	0.058	172
PisoF_02323	hypothetical protein	0.067	42	0.070	43	0.063	39
PisoF_02324	conjugal transfer protein TrbN	0.037	20	0.051	27	0.042	22
PisoF_02325	type IV secretion system protein DotD	0.071	36	0.050	25	0.043	21
PisoF_02326	type IV secretion system protein DotC	0.051	49	0.060	58	0.043	41
PisoF_02327	type IV secretion protein DotB	0.110	132	0.089	107	0.085	102
PisoF_02328	type IV secretion system protein IcmT	0.056	15	0.077	21	0.078	21
PisoF_02329	type IV secretion system protein IcmP/DotM	0.082	109	0.058	76	0.052	69
PisoF_02330	disulfide isomerase/thiol-disulfide oxidase	0.030	29	0.036	35	0.029	28
PisoF_02331	hypothetical protein	0.031	25	0.035	28	0.031	24
PisoF_02332	hypothetical protein	0.009	8	0.002	2	0.006	5
PisoF_02333	hypothetical protein	0.034	20	0.024	14	0.020	11
PisoF_02334	hypothetical protein	0.059	50	0.035	30	0.061	52
PisoF_02335	hypothetical protein	0.034	12	0.048	17	0.047	17
PisoF_02336	hypothetical protein	0.060	36	0.057	34	0.072	43
PisoF_02337	type IV secretion system protein DotA-like protein	0.068	244	0.070	251	0.062	221
PisoF_02338	hypothetical protein	0.101	43	0.110	46	0.113	47
PisoF_02339	hypothetical protein	0.023	10	0.026	12	0.039	18
PisoF_02340	hypothetical protein	0.078	141	0.073	132	0.053	95
PisoF_02341	hypothetical protein	0.062	27	0.053	23	0.042	18
PisoF_02342	hypothetical protein	0.101	127	0.105	132	0.106	133
PisoF_02343	hypothetical protein	0.096	51	0.099	53	0.103	55

Locus_tag	Description	Liquid		Solid		Mixed	
		UID	UIC	UID	UIC	UID	UIC
PisoF_02344	hypothetical protein	0.073	36	0.065	32	0.081	40
PisoF_02345	type IV secretion system protein DotU	0.110	78	0.121	86	0.133	94
PisoF_02346	hypothetical protein	0.064	22	0.086	30	0.059	21
PisoF_02347	hypothetical protein	0.033	20	0.034	21	0.039	24
PisoF_02348	hypothetical protein	0.065	48	0.060	45	0.077	57
PisoF_02349	hypothetical protein	0.104	47	0.116	52	0.088	40
PisoF_02350	hypothetical protein	0.050	21	0.073	31	0.061	26
PisoF_02351	hypothetical protein	0.009	12	0.008	10	0.010	14
PisoF_02352	peptidase M23	0.025	29	0.011	13	0.013	14
PisoF_02353	hypothetical protein	0.040	48	0.027	32	0.035	41
PisoF_02354	hypothetical protein	0.026	30	0.016	18	0.012	14
PisoF_02355	hypothetical protein	0.044	33	0.030	23	0.025	19
PisoF_02356	hypothetical protein	0.072	60	0.065	54	0.070	59
PisoF_02357	type IV secretion protein IcmW	0.074	38	0.069	36	0.054	28
PisoF_02358	hypothetical protein	0.187	401	0.176	379	0.169	364
PisoF_02359	nuclease	0.092	71	0.079	61	0.081	63
PisoF_02360	DNA helicase II	0.165	305	0.145	268	0.151	280
PisoF_02361	putative transcriptional regulator	0.100	20	0.083	17	0.093	19
PisoF_02362	Type I restriction enzyme EcoKI M protein	0.161	250	0.129	201	0.133	207
PisoF_02363	Type I restriction enzyme specificity protein MPN_089	0.100	115	0.079	91	0.069	79
PisoF_02364	anticodon nuclease	0.070	83	0.048	58	0.048	58
PisoF_02365	Type I restriction enzyme EcoR124II R protein	0.126	392	0.119	368	0.117	364
PisoF_02366	putative P-loop ATPase	0.138	248	0.135	242	0.118	212
PisoF_02367	hypothetical protein	0.069	33	0.062	30	0.046	22
PisoF_02368	7-cyano-7-deazaguanine synthase	0.109	143	0.103	135	0.091	119
PisoF_02369	hypothetical protein	0.107	28	0.073	19	0.105	27
PisoF_02370	integrase	0.093	91	0.096	94	0.087	85
PisoF_02371	hypothetical protein	0.182	65	0.200	72	0.172	62
PisoF_02372	hypothetical protein	0.098	137	0.088	123	0.109	153
PisoF_02373	prophage CP4-57 integrase	0.146	184	0.155	195	0.160	201
	Total number of UIC in the I-GI[3]		5283		4912		4793

[1] UID: Unique insertion density

[2] UIC: Unique insertion count

[3] Normalized number of UIC using the number of unique insertion sites from Table 2.

References

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