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Supporting information

55.1, a gene of unknown function of phage T4 impacts on Escherichia coli folate metabolism and blocks DNA repair by the NER

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Supplementary Experimental procedures

E.coli strain construction. YM21 was obtained by phage P1*vir*-mediated transduction (Miller, 1992) of NM538 (Frischauf *et al*., 1983) with a CAG12169 (Singer *et al*., 1989) lysate and selecting for Tc^r recombinants. YM22 was obtained by transduction of DHB3 with a YM21 lysate and screening Tc^r recombinants for *supF* using a *31*(*am*) T4 strain. YM38 was obtained by transduction of DHB3 with a K996 (*E. coli* genetic stock center; (Mount *et al*., 1972)) lysate and screening Tc^r recombinants for UV sensitivity conferred by *lexA3*(Ind^-); YM37, a UV resistant Tc^r recombinant, was chosen as $lexA^+$ control strain. Deletions of *uvrA*, *uvrB*, *uvrC*, and *uvrD* from the Keio collection (Baba *et al*., 2006) were transferred by transduction to DHB3 to generate YM42, YM43, YM44, YM45 respectively.

T4 strain construction. A *55.1* null mutant (ATG -> GTT mutation at T4 coordinates 40'456-40'454 that destroy the *55.1* initiation codon and generates a HpaI site) was constructed using the T4 I/S system (Kreuzer & Selick, 1994, Selick *et al*., 1988). A 632 bps fragment with the full-length genes *55.2* and *55.1* and containing the above-described mutation was generated by PCR site-directed mutagenesis (Ho *et al*., 1989) using primer 5'-GTTAATAAGAGCTTCGTTAACAATTATACCTTAG and 5'-CTAAGGTATAATTGTTAACGAAGCTCTTATTAAC (mutation underlined). The fragment was cloned between the EcoRI and SalI site of pBSPL0+ and the construction was verified by sequencing. The mutation carried by the recombinant plasmid was then transferred to the T4 K10 genome. The presence of the deletion was verified by PCR followed by HpaI digestion. In a pBAD vector expressing *55.1*, the ATG -> GTT mutation completely abolished all *55.1* induced phenotypes.

Plasmid construction. pBAD22Kn (Ap^r, Kn^r) was constructed by ligating NsiI-digested pBAD22 with a PstI fragment of pUC4K (GE Healthcare) containing a Kn^r cassette. pBAD18Kn was obtained by ligating a SalI-digested fragment of pUC4Kn into SalI-digested pBAD18. pDB21 is derived from pBAD18Kn and contains a 1486 bps insert of T4 genome (position 39'453 to 40'939 on the T4 genome, GenBank: AF158101.6); the insert contains genes *55*, *55.1* and *55.2*. pDB2101, containing *55*, was obtained by digesting pDB21 with BglII and NheI, filling in with Klenow fragment and self ligating. pDB2112, pDB2113, pDB2114 expressing respectively *55.2-55.1* (position 40'785 to 40'182), *55.1* (position 40'456 to 40'182) and *55.2* (position 40'785 to 40'458) under the control of the P_{BAD} promoter were constructed

by PCR amplification and cloning between the NcoI and XbaI sites of pBAD22Kn. pYM2 was derived from pDB2112 by deletion of P_{BAD} and part of *araC* using NheI/AflII digestion followed by Klenow fragment fill-in and self-ligation. pYM3 was constructed by digesting pYM2 with NdeI and HincII, followed by Klenow fill-in, isolating a 449 bps fragment containing the 3' end of *55.2* and 5' end of *55.1*, cloning into the SmaI site of pBluescript II SK (Stratagene), and selecting a clone whose T4 fragment orientation was reverse to the *lacZ* α fragment. pYM5 was obtained by BclI/EcoRI digestion of pDB2112, Klenow fragment fill-in and self ligation. pYM18, a pDB2112 derivative bearing an amber stop codon instead of the Tyr codon at position 14 of gp55.1, was generated by PCR site-directed mutagenesis (Ho et al., 1989) using primer 5'-TCTCGCTGGGTAGGAAGTAAATACAAATGG and 5'-CCATTTGTATTTACTTCCTACCCAGCGAGA (mutation underlined). pJM2, a pDB2113 derivative expressing a N-terminal His_6 tagged gp55.1, was generated by inserting a His_6 tag containing, NcoI-digested, Klenow fragment treated, BssHII-digested fragment of pBAD22-HisEYG (Collinson *et al*., 2001) into a EcoRI-digested, Klenow fragment treated, BssHII-digested pDB2113 vector. pDB2112-33, pDB2113-33, pDB2114-33, pYM2-33 and pYM5-33 (all Cm^r) were derivated from their parent plasmids by ligating the EcoRV/XbaI-digested fragments with EcoRV/XbaI-digested pBAD33 vector (p15A origin). pJM1-33 and pJM2-33 derivated from their parent plasmids by ligating an EcoRV/SphI-digested fragment with a EcoRV/SphI-digested pBAD33 vector. pBAD33-K, pJM1-33-K, and pYM2-33-K were constructed by ligating an EcoRI-digested, Klenow filled-in fragment of pUC4K (GE Healthcare Life Science), which contain the Kn^r cassette, into the unique, Klenow blunted, NcoI site of their parent plasmid. pYM15 (Ap^r) was constructed by PCR amplification of *folD* with its promoter (position 557'113 to 556'087 on the *E. coli* K12 MG1655 genome, GenBank: U00096.2) and cloning into XbaI, HindIII sites of pMPM-A2; pYM15 can express *folD* from two promoters P_{Lac} and P_{FolD}. pBAD-*uvsW** expressing both *uvsW* and *uvsW.1* (coordinates 112'635 to 114'567 on the T4 genome) under the control of the P_{BAD} promoter was constructed by subcloning a BspHI/KpnI-digested fragment from one of the original library clones into NcoI and KpnI sites of pBAD22Kn (this plasmid contain 41 bps of the *uvsW* 5' UTR). pYM51 was constructed by PCR amplification of *uvsW* without *uvsW.1* (but including the 5'UTR found in pBAD-*uvsW**) and cloning into the NheI, NcoI sites of pBAD22Kn. pYM52 and pYM53 were constructed by cloning XbaI/EcoRV

fragments of pKCK43 and pKCK41 (Carles-Kinch *et al*., 1997), which contain *uvsW* K141R and *uvsW* respectively (both also contain *uvsW.1*), into the EcoRI, Klenow filled-in/NheI sites of pBAD22Kn (these plasmids contain a 35bps 5' UTR derived from pET11d (NEB) before the *uvsW* initiation codon). All PCR generated constructions were verified by sequencing.

Genomic libraries. A T4 genomic DNA library (gift from Dr. D. Ang) was constructed by ligating Tsp509I-partially digested 2-4 kbs $T4^+$ genomic DNA into EcoRI-digested pBAD18Kn. An *E. coli* genomic DNA library (gift from Dr. P. Genevaux*)* was constructed by ligating 1.5-5 kbs genomic DNA fragments of CG3211 (MC4100 *ΔdnaKdnaJ Δtig)* into the BamHI site of pMPM-A2.

Immunoblotting. Samples were separated in 15% SDS-polyacrylamide gels and proteins were transferred to nitrocellulose membranes (Protran BA 85, Schleicher & Schuell, Keene, NH). Proteins were detected with chemiluminescence using mouse monoclonal anti-LexA antibody (P06004, Dualsystems Biotech, Schlieren, CH) and horseradish peroxidase-linked anti-mouse goat antibody (Bio-Rad). Quantification was performed with a FujjiFilm LAS-4000mini and image analysis with the MultiGauge (v.3) software (Fujifilm LifeScience). Signals were calibrated using a standard curve of diluted control samples.

β-galactosidase assay. Cells were grown in LB media to mid-logarithmic phase. Aliquots were withdrawn from each culture, cooled on ice, resuspended in cold MOPS buffer (66 mM MOPS, 83 mM NaCl, 16 mM NH_4Cl, 10 mM $MgCl_2$, pH7.2) and permeabilized by chloroform/SDS treatment. The β-galactosidase activity was determined by the rate of ONPG hydrolysis as described previously (Miller, 1992).

Mutation rate analysis. Mutation rate (μ) analysis was performed according to published methods (Foster, 2006, Rosche & Foster, 2000). Individual 1.8 ml LB cultures were inoculated with 0.2 ml of a 10^{-5} dilution of a saturated LB culture (total V = 2 ml, inoculum size $\approx 10^4$ cells). Cells were grown 14 h at 37°C and 0.2 ml aliquots of each culture where spread on fresh LB rifampicin plate (100 μg ml^{-1}); 0.1 ml aliquots of 10^{-6} dilutions were spread on standard LB plate to assess total cfus. Cfus were counted after over-night incubation and μ was derived from the median number of mutants using the Jones Median Estimator (Crane *et al*., 1996, Jones, 1994).

TABLE S1. Plasmids

Plasmid	Description	Reference
pBAD18, pBAD22	Vectors with pBR322 replication origin, P_{BAD} promoter and *araC*, Ap^r	(Guzman *et al*., 1995)
pBAD18Kn	pBAD18 with additional Kn^r	this study
pBAD22Kn	pBAD22 with additional Kn^r	this study
pBAD33	Vector with pACYC184 replication origin, P_{BAD} and *araC*, Cm^r	(Guzman et al., 1995)
pBAD33-K	Vector with pACYC184 replication origin, P_{BAD} and *araC*, Kn^r	this study
pBAD-*uvsW**	pBAD22Kn expressing *uvsW* and *uvsW.1* from P_{BAD}	this study
pCA24N(-)	Vector with pBR322 replication origin, $P_{T5\text{-}lac}$ (IPTG-inducible T5 promoter) and $lacI^q$, Cm^r	(Kitagawa *et al*., 2005)
pCA24N-*adK*	pCA24N(-) expressing His-*adK* from $P_{T5\text{-}lac}$	(Kitagawa et al., 2005)
pCA24N-*folD*	pCA24N(-) expressing His-*folD* from $P_{T5\text{-}lac}$	(Kitagawa et al., 2005)
pCA24N-*uvrA*	pCA24N(-) expressing His-*uvrA* from $P_{T5\text{-}lac}$	(Kitagawa et al., 2005)
pCA24N-*uvrB*	pCA24N(-) expressing His-*uvrB* from $P_{T5\text{-}lac}$	(Kitagawa et al., 2005)
pCA24N-*uvrC*	pCA24N(-) expressing His-*uvrC* from $P_{T5\text{-}lac}$	(Kitagawa et al., 2005)
pCA24N-*uvrD*	pCA24N(-) expressing His-*uvrD* from $P_{T5\text{-}lac}$	(Kitagawa et al., 2005)
pDB21	pBAD18Kn expressing *55.2*, *55.1* and *55* from P_{BAD}	this study
pDB2101	pBAD18Kn expressing *55* from P_{BAD}	this study
pDB2112	pBAD22Kn expressing *55.2* and *55.1* from P_{BAD} and *55.1* from P_{T1+2}	this study
pDB2112-33	pBAD33 expressing *55.2* and *55.1* from P_{BAD} and *55.1* from P_{T1+2}	this study
pDB2113	pBAD22Kn expressing *55.1* from P_{BAD}	this study
pDB2113-33	pBAD33 expressing *55.1* from P_{BAD}	this study
pDB2114	pBAD22Kn expressing *55.2* from P_{BAD}	this study
pDB2114-33	pBAD33 expressing *55.2* from P_{BAD}	this study
pJM1-33-K	pBAD33-K expressing *55.1*-HA (C-terminal tag) from P_{BAD}	this study
pJM2-33	pBAD33 expressing His-*55.1* (N-terminal tag) from P_{BAD}	this study
pMPM-A2	Vector with pBR322 replication origin, Ap^r	(Mayer, 1995)
pYM2	pBAD22Kn without the P_{BAD}, expressing *55.1* from P_{T1+2}	this study
pYM2-33-K	pBAD33-K without the P_{BAD}, expressing *55.1* from P_{T1+2}	this study
pYM3	pBluescript II SK with a NdeI, HincII fragment containing the 3' end of gene *55.2* and the 5' end of gene *55.1*	this study
pYM5	pBAD22Kn expressing *55.1* from P_{BAD} and from P_{T1}	this study

pYM5-33	pBAD33 expressing *55.1* from P_{BAD} and from P_{T1}	this study
pYM15	pMPM-A2 expressing *folD* from the P_{Lac} and P_{FolD} promoters	this study
pYM18	pDB2112 with a T->G mutation that generates an amber stop codon at amino acid position 14 of *55.1*	this study
pYM51	pBAD22Kn expressing *uvsW* from P_{BAD}	this study
pYM52	pBAD22Kn expressing *uvsW* K141R and *uvsW.1* from P_{BAD} (5' UTR of pET11d (NEB))	this study
pYM53	pBAD22Kn expressing *uvsW* and *uvsW.1* from P_{BAD} (5' UTR of pET11d (NEB))	this study

Supplementary Figures

Figure S1

Map of the plasmids used in this study. P_{T1} and P_{T2}: T4 early promoters; P_{BAD} : arabinose inducible promoter. The inserts are drawn to scale.

Figure S2

Two promoters located in the *55.2* coding sequence direct *55.1* expression and UV sensitivity. UV viability assay. Exponentially growing DHB3 cells transformed with pBAD22Kn (vector), pDB2112 (*55.1+2*), or pYM5 (P_{T1}*55.1*) were submitted to the indicated dose of UV light and diluted aliquots were plated on LB plates to determine cfus. Data are means and standard errors for three independent cultures.

Figure S3

uvsW.1 enhances the suppression of *55.1*-induced toxicity and UV sensitivity by *uvsW*. (A) DB503 cells transformed with pBAD33 (vector) or pDB2113-33 (*55.1*) and one of the compatible plasmids, pBAD22K (vector) or pYM51 (*uvsW*), were streaked on LB plates with or without 0.2% arabinose. (B) UV sensitivity assay. DHB3 cells were transformed with pBAD33 (–) or pYM5-33 (+) and one of the compatible plasmids, pBAD22K (vector), pBAD-*uvsW* (uvsW+uvsW.1)*, or pYM51 (*uvsW*). Dilutions of overnight cultures were streaked on LB plates without arabinose before irradiation with the indicated doses of UV light.

Figure S4

High-level expression of *uvsW* and *uvsW.1* reduces the plasmid copy number (PCN) but *uvsW* suppresses *55.1* toxicity independently of this effect. (A) PCN determination. DHB3 cells were transformed with pBAD33 and compatible pBAD22Kn (–) or pBAD-*uvsW* (+). Exponentially growing cells ($A_{600\ nm}$ = 0.4) were diluted 1:10 in pre-warmed LB media and treated or not with 0.2% arabinose for 3 h. Plasmid DNA was extracted from equal amount of each culture and linearized with EcoRV. DNA was quantified by agarose gel electrophoresis and ethidium bromide staining. The positions of migration of the linearized plasmids are indicated. (B). DHB3 cells were transformed with pBAD101 (vector) or pDB2113-101 (*55.1*) and compatible pBAD22Kn (vector) or pBAD-*uvsW** (*uvsW**). The indicated dilutions of overnight cultures were spotted on LB plates minus or plus 0.2% arabinose.

Figure S5

Scheme of the folate cycle of *E. coli*. The two reactions catalyzed by FolD are highlighted in red. The enzymes that catalyses the formation of THF are in black; enzymes that are implicated in amino acids metabolism in blue; enzymes that are implicated in nucleotides anabolism in purple; Fmt catalyses the formation of the initiator $tRNA^{fMet}$. FolA is inhibited by trimethoprim (TMP). The catalytic activity of FolM is at least 4 times lower than that of FolA (Leduc *et al*., 2007). *Adapted from KEGG pathway for E. coli MG1655 (http://www.genome.jp/dbget-bin/www_bget?pathway:map00670)*.

Figure S6

55.1 increases the sensitivity of bacteria to mitomycin C and hydroxyurea. (A) DNA damaging agents disc test. Overnight cultures of DHB3 cells transformed with pBAD22Kn (vector) or pYM5 (P_{T1}*55.1*) were used to measure the sensitivity to H_2O_2 (H2O2, 0.5 mg), hydroxyurea (HU, 1.9 mg), or mitomycin C (MMC, 5 μg). (B). Antibiotic disc test. Overnight cultures of the above-indicated strains were used to measure the sensitivity to kasugamycin (KG, 665 μg), tetracycline (TC, 24.8 μg), chloramphenicol (CM, 99 μg), or spectinomycin (SPC, 330 μg). Data represent mean area of inhibition and standard errors of three independent cultures.

Figure S7

Arabinose induced expression of His-*55.1* from pJM2-33 is not toxic but leads to UV sensitivity. DHB3 were transformed with pBAD33 (vector) or pJM2-33 (His-*55.1*) and cultivated overnight in LB media plus or minus 0.2% arabinose. (A) appropriate dilutions of the overnight cultures were spotted on LB plates. (B) Single dilutions of the overnight cultures were plated on LB plates plus or minus 0.2% arabinose before irradiation with the indicated doses of UV light.

Figure S8

55.1 further increases the UV sensitivity of bacteria unable to induce the SOS response. (D) UV sensitivity assay. Strains YM37 (DHB3 *malE300*::Tn*10;* control) and YM38 (DHB3 *malE300*::Tn*10 lexA3*; *lexA3*(Ind^-)) were transformed with pBAD22Kn (–) or pYM5 (+) and dilutions of overnight cultures were streaked on LB plates without arabinose before irradiation with the indicated doses of UV light. Bacteria carrying the *lexA3* cleavage defective allele of *lexA* are defective in SOS induction (Mount et al., 1972).

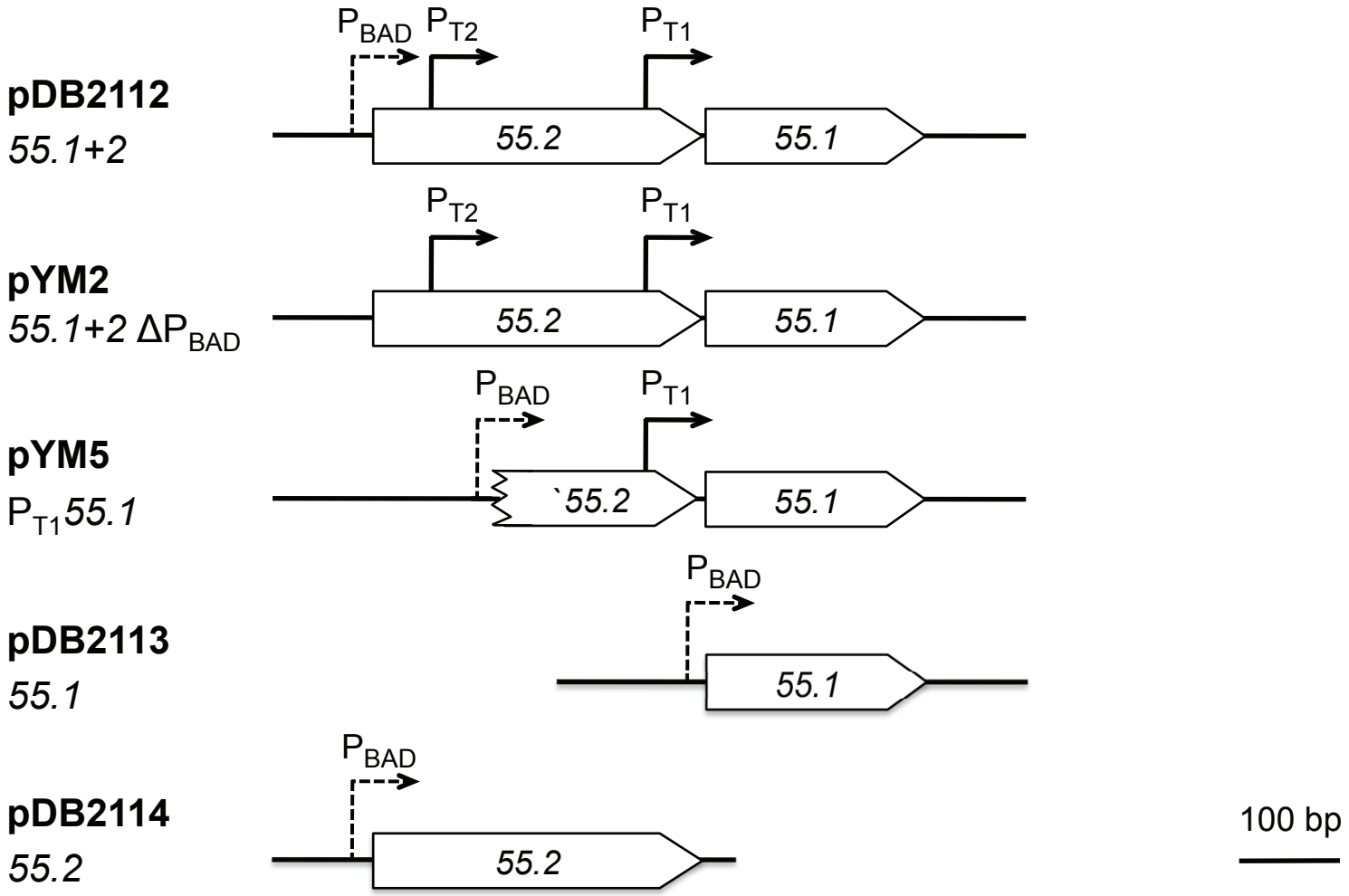
Figure S9

Low-level expression of *55.1* leads to a moderate activation of the SOS response. (A) β-galactosidase assay. Exponentially growing JM36612 (F' *recN*::*lacZ*, from Miller JH) cells transformed with plasmids pBAD22Kn (vector), pDB2113 (*55.1*), pYM5 (P_{T1}*55.1*), or pDB2112 (*55.1+2*) were assayed for β-galactosidase activity. Values of control cells irradiated with 32 J/m^2 of UV light and grown 1 h afterwards are included. Data are means and standard errors for three independent cultures. (B) Analysis of LexA steady-sate levels. Three independent cultures of DHB3 cells transformed with pBAD22Kn (vector), pYM5 (P_{T1}*55.1*), or pDB2112 (*55.1+2*) were grown to mid-log phase in LB medium, lyzed in SB+ buffer and analyzed by Western blot with an anti-LexA monoclonal antibody. As a control, a culture was irradiated with 16 J/m^2 of UV light and grown for an additional 30 min. Upper panel, Western blot; lower panel, quantification of the Western blot, data are means and standard errors. (C) Mutation rate analysis. Independent overnight cultures of DHB3 cells transformed with pBAD22Kn (vector, n=55) or pYM5 (P_{T1}55.1, n=55) were plated on LB plates supplemented with rifampicin to determine the number of spontaneous Rif^r mutants. As a positive control, YM4 cells (DHB3 *Δdam-16*::*kan*, n=10) were included. The graph depicts the number of Rif^r colonies in 0.2 ml of each culture; the median is indicated by a line. Mutation rates (μ) were calculated as described (n.d. not determined).

Figure S10

His-tagged proteins are efficiently expressed and pulled-down by Ni^{2+} NTA agarose beads. Exponentially growing DB503 cells transformed with pBAD33-K (–) or pJM-33-K (+) and one of the compatible plasmids, pCA24N(-) (vector), pCAN24N-*folD* (*folD*), pCAN24N-*uvrA* (*uvrA*), pCAN24N-*uvrB* (*uvrB*), pCAN24N-*uvrC* (*uvrC*), or pCA24N-*adK* (His-*adK*) were induced with 100μM IPTG and 0.2% arabinose and cross-linked with 0.6% formaldehyde. His-tagged proteins were pulled-down on Ni-NTA agarose beads. Whole cell extracts (W) and eluted proteins (P) were analyzed by Coomassie staining of a 12% SDS-PAGE gel. Uncross-linked gp55.1-HA migrates with the migration front.

Figure S1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55.2
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55.1+2 ΔP BAD
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P T1
55.2
55.1
pYM5
P T1 55.1
P BAD
P T1
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55.2
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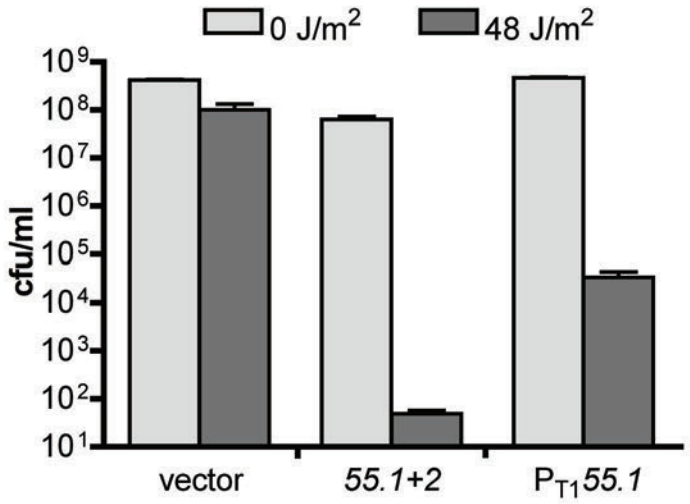
Figure S2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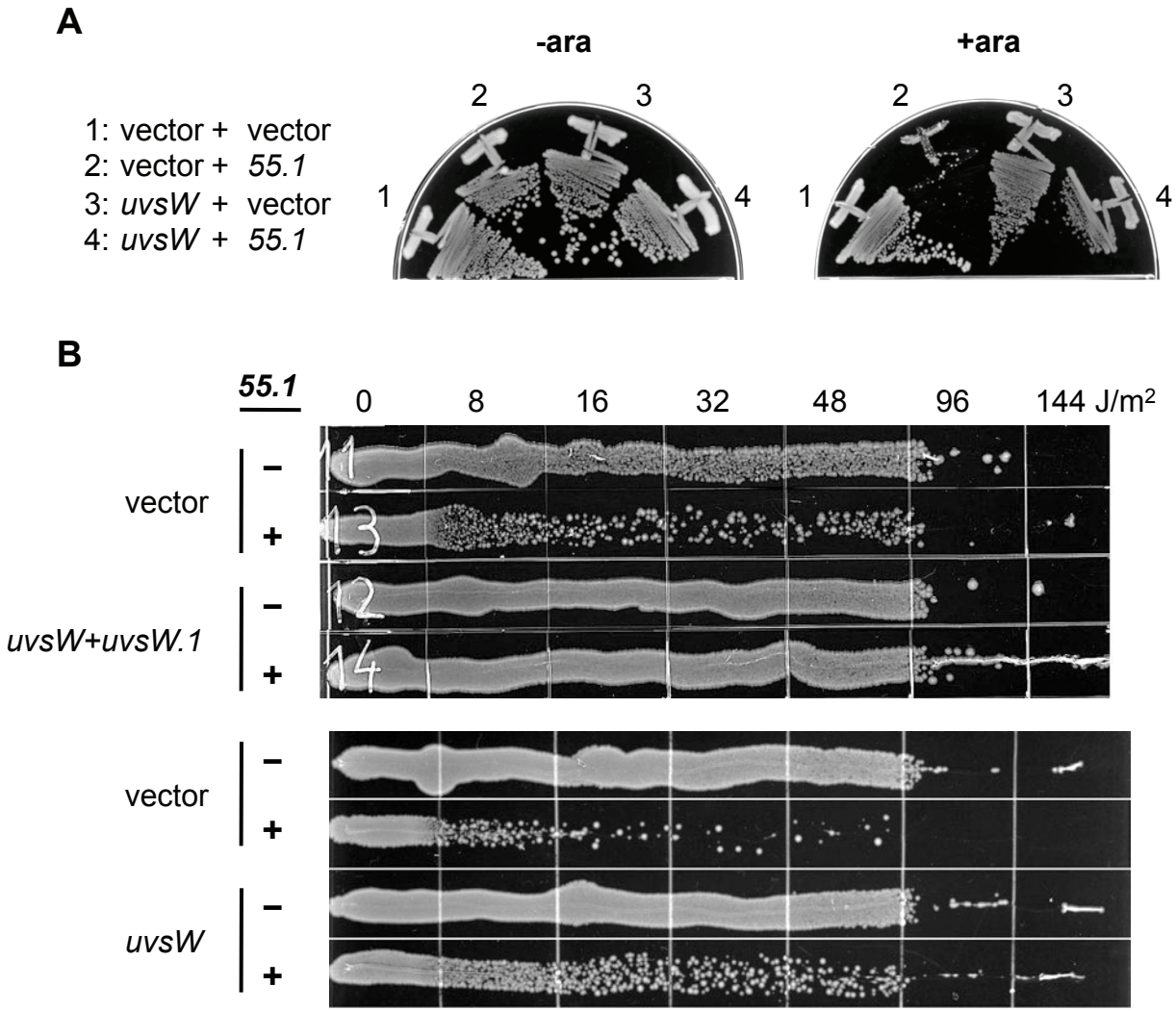
vector
55.1+2
PT1 55.1

Figure S3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+ara
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2
3
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B
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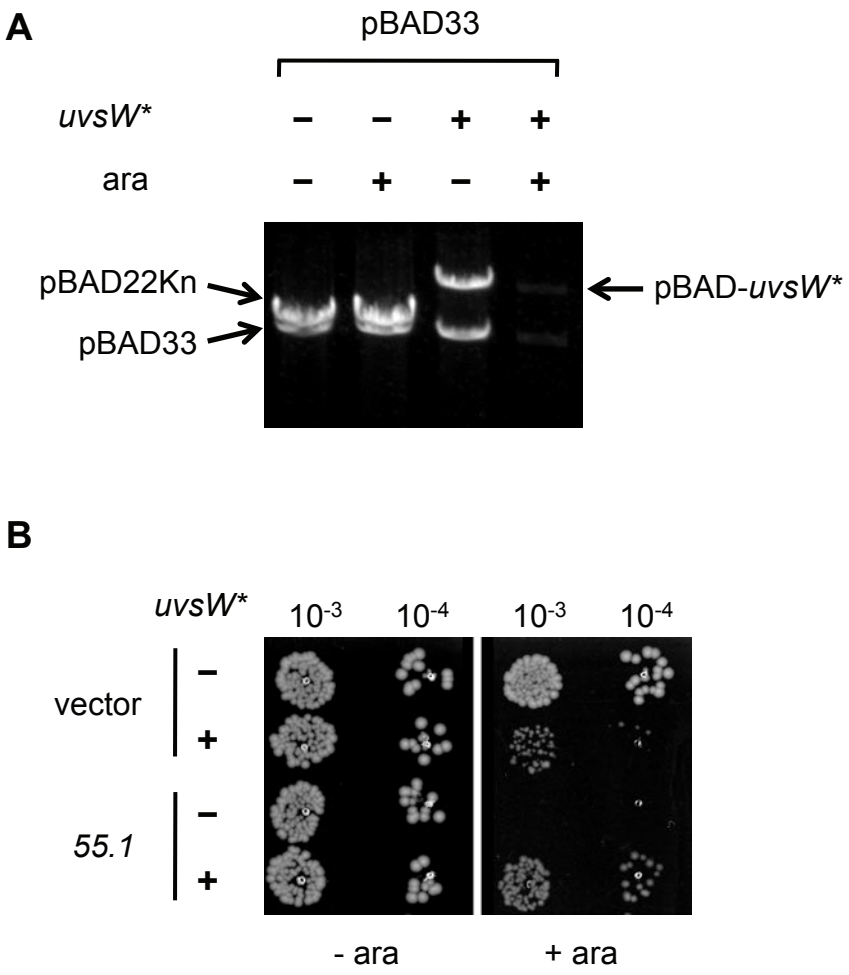
–
+

Figure S4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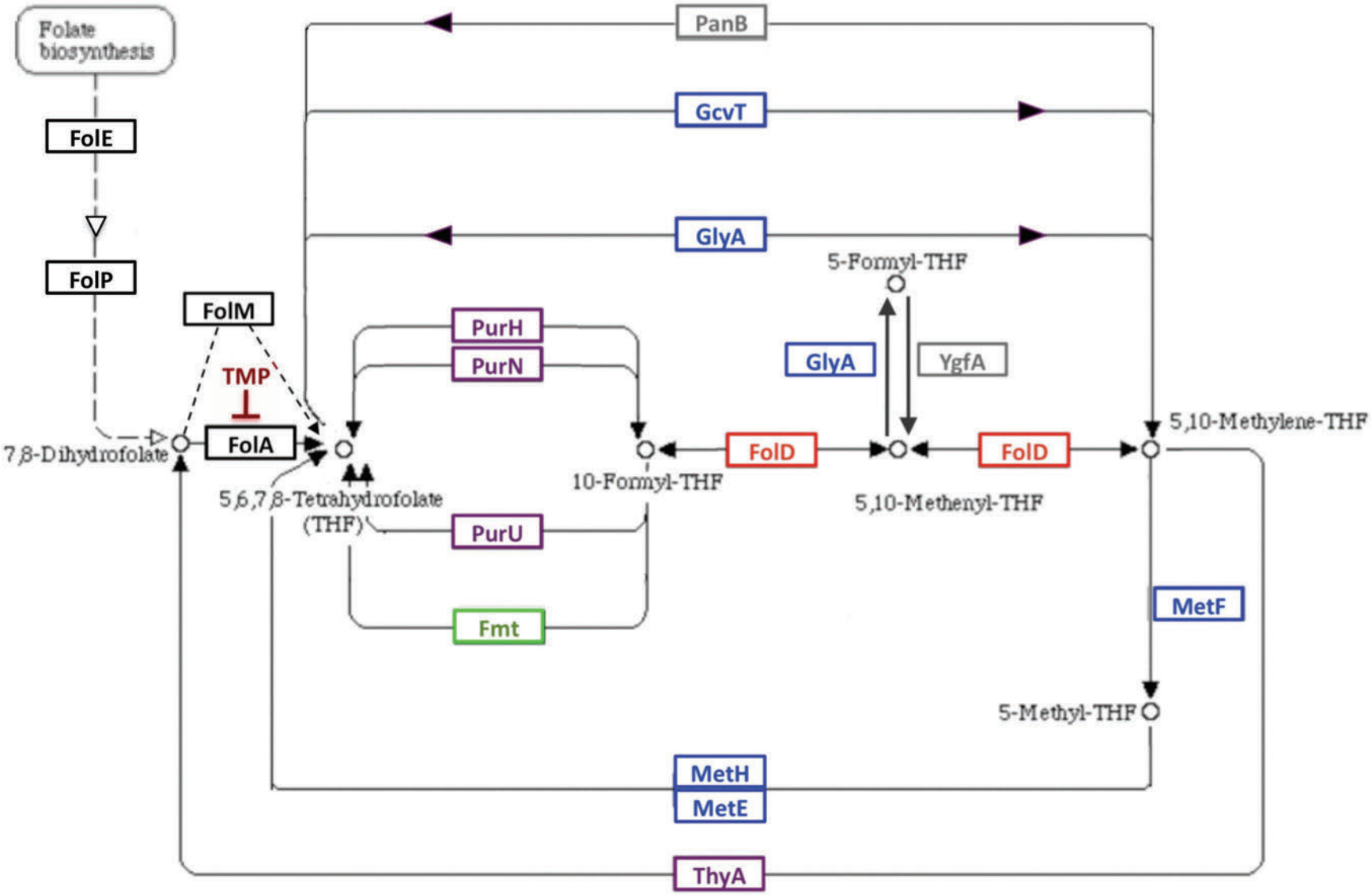
– +
- ara
+ ara

Figure S5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GlyA
YgfA
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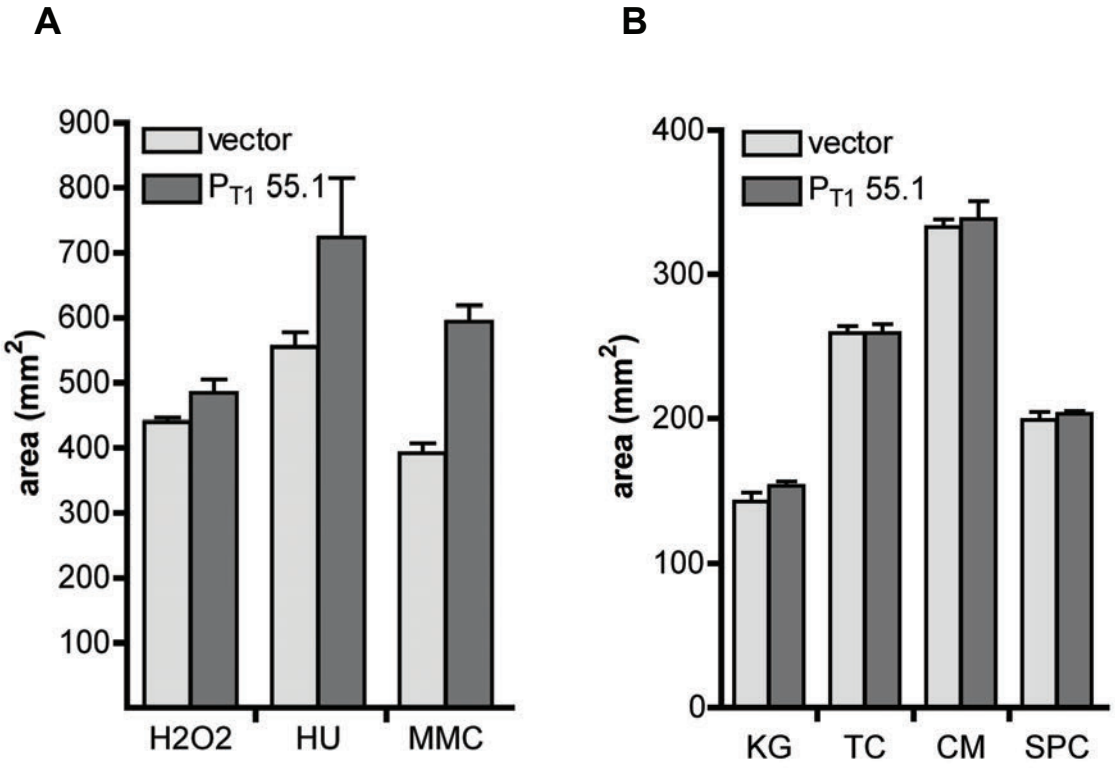
Figure S6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CM
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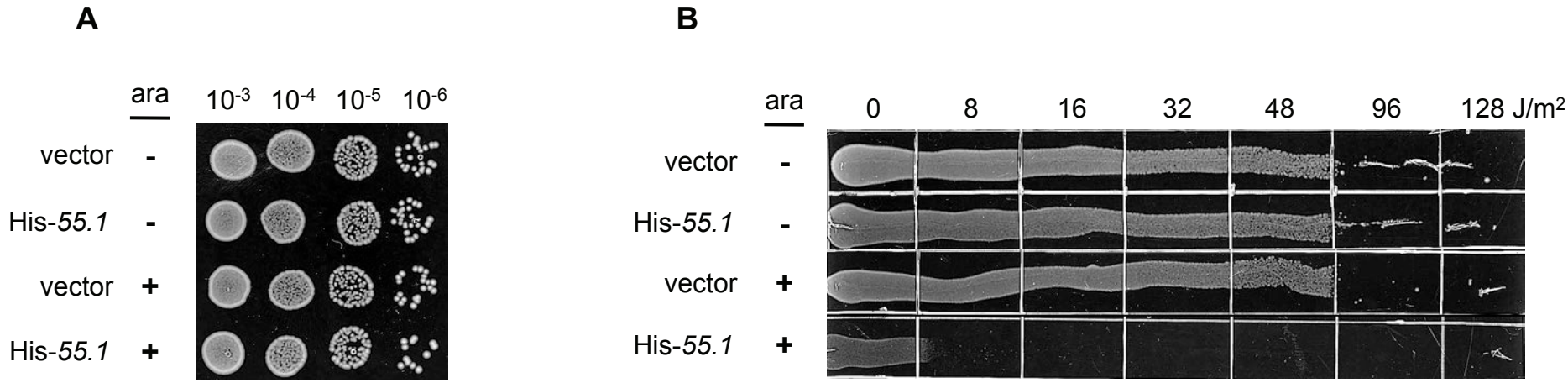
Figure S7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His-55.1 -
vector +
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vector +
His-55.1 +

Figure S8

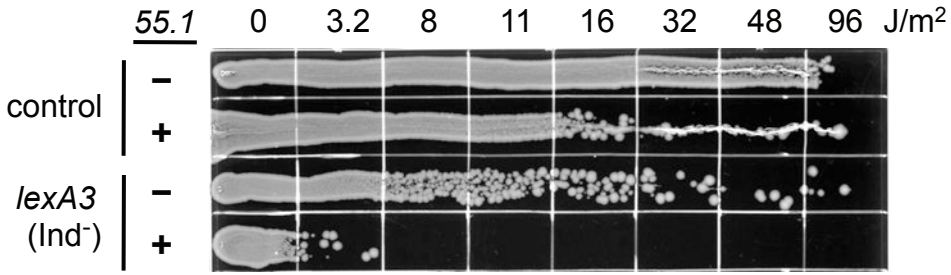
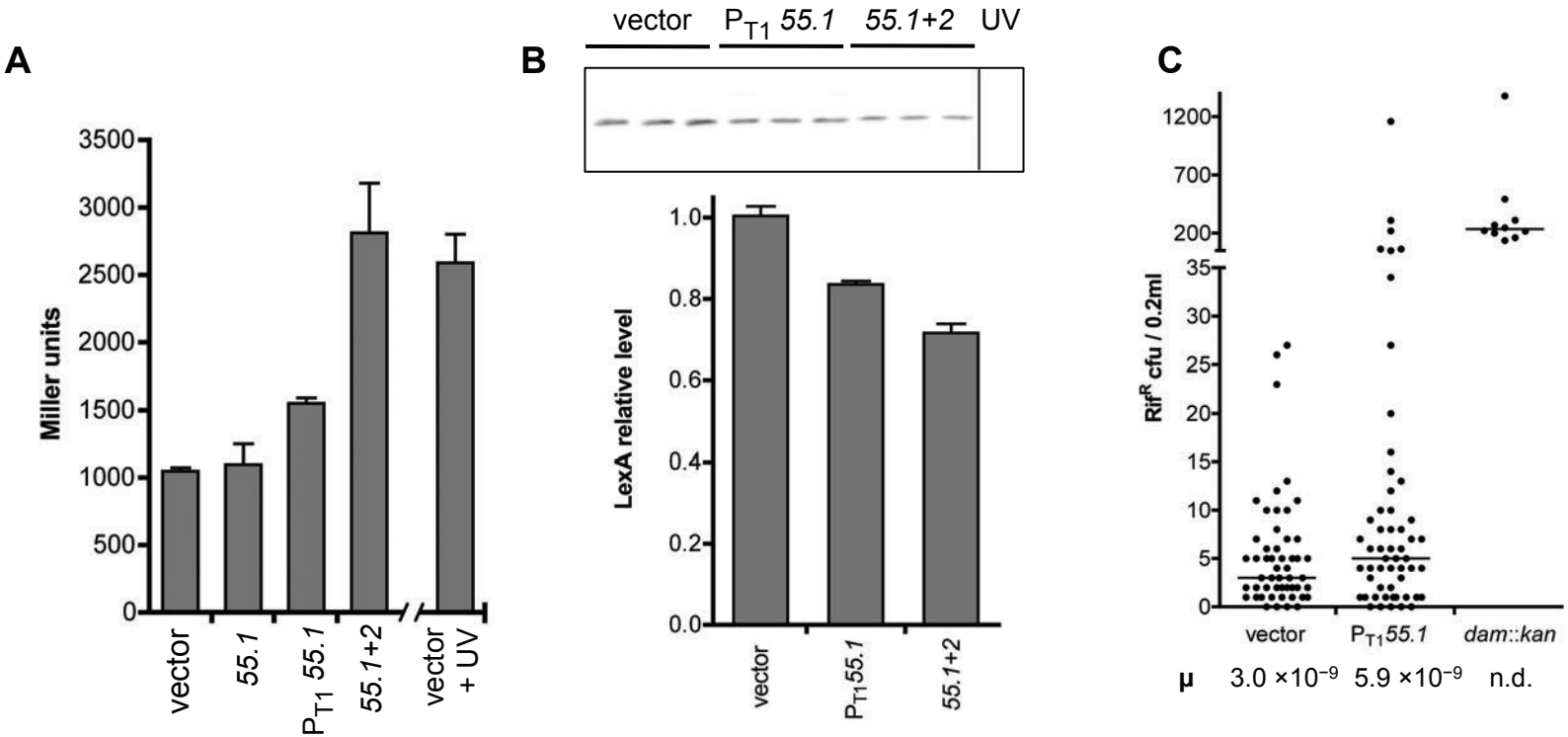


Figure S9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1000
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vector
P_T1 55.1
55.1+2
UV
LexA relative level
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0.6
0.4
0.2
0.0
vector
P_T1 55.1
55.1+2
C
Rif^R cfu / 0.2ml
1200
700
200
35
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20
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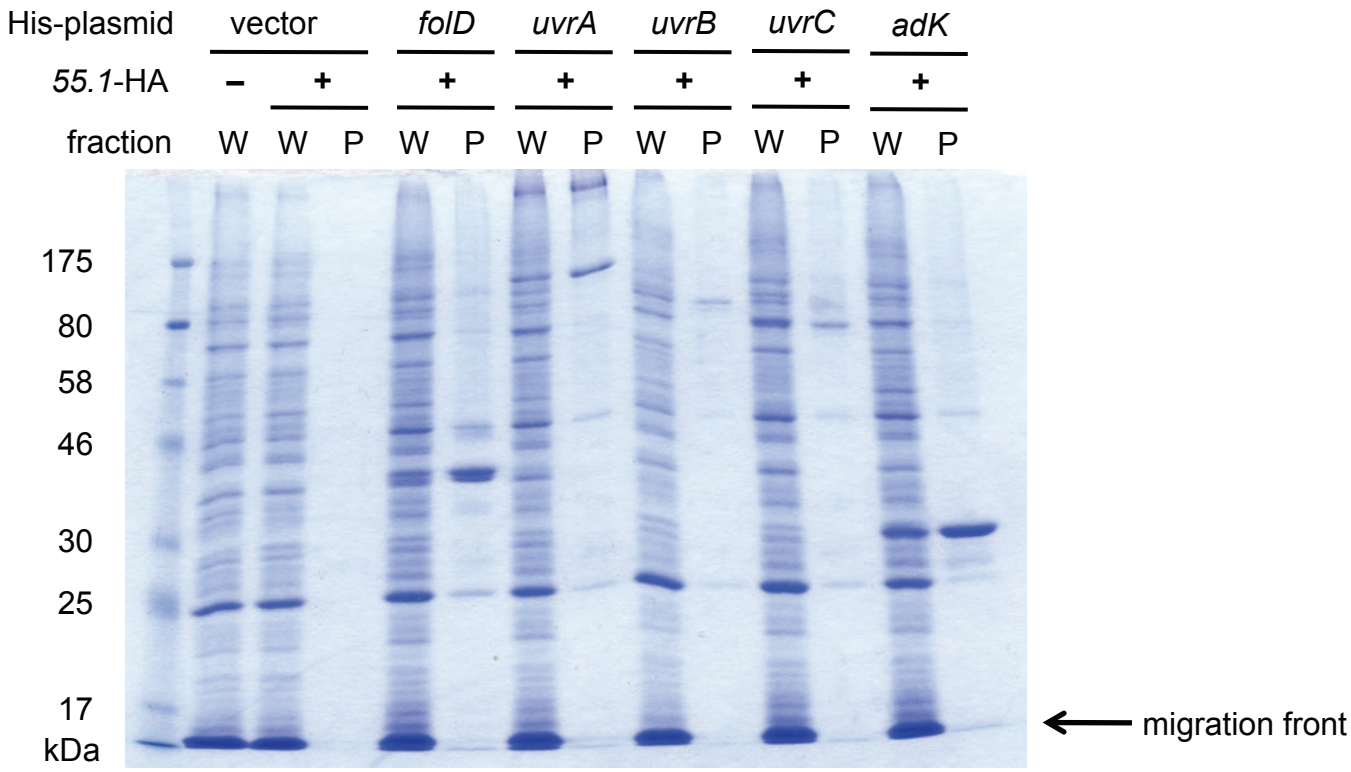
0
vector
P_T1 55.1
dam::kan
μ
3.0 ×10⁻⁹
5.9 ×10⁻⁹
n.d.

Figure S10


His-plasmid
vector
folD
uvrA
uvrB
uvrC
adK
55.1-HA
–
+
+
+
+
+
+
fraction
W W P W P W P W P W P W P
175
80
58
46
30
25
17
kDa
migration front

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