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Supplemental information

Cyanobacterial Argonautes and Cas4 family nucleases cooperate to interfere with invading DNA

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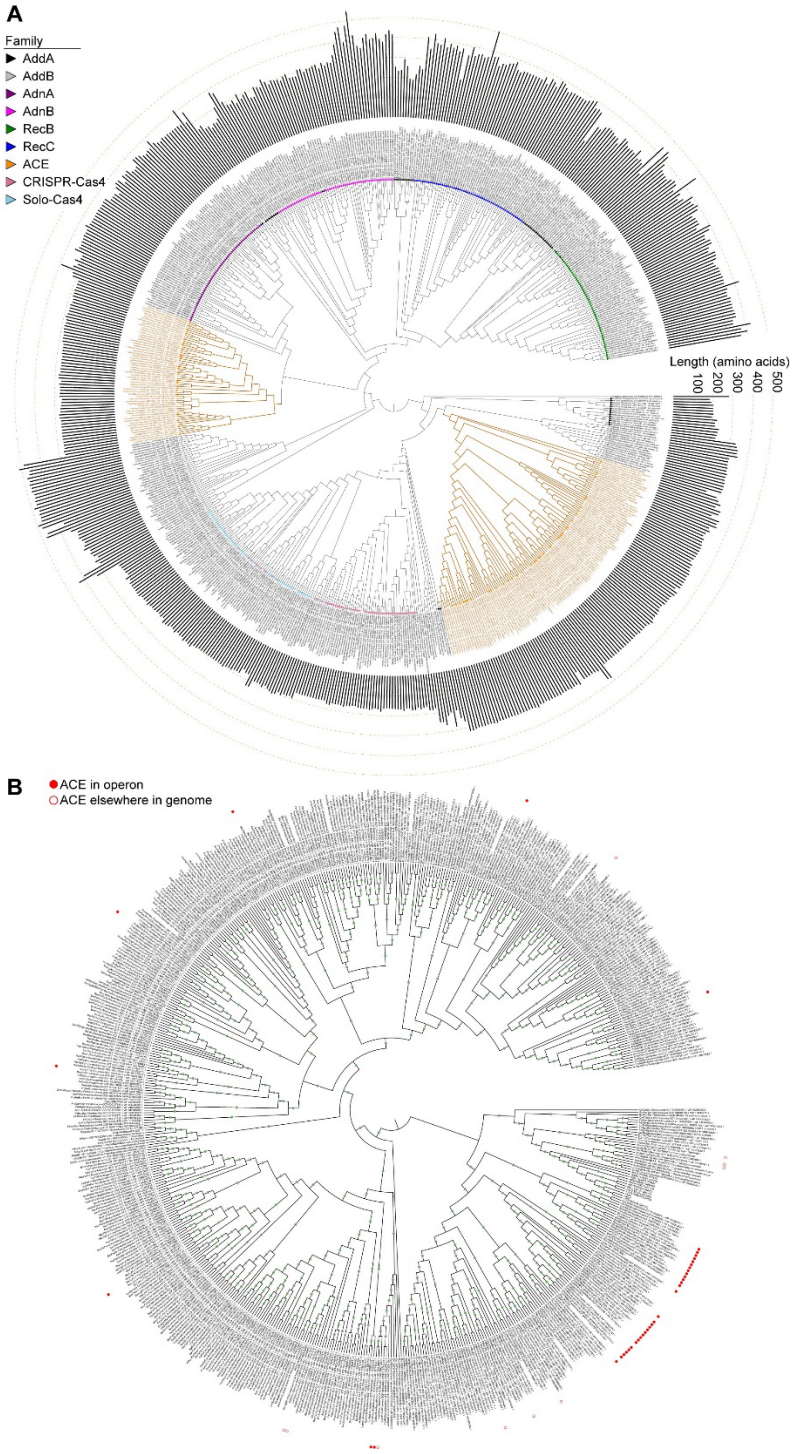

Figure S1. Phylogeny of Cas4 family proteins and associated pAgos. Related to Figure 1.

(**A**) Maximum likelihood-based phylogenetic tree containing Cas4 family proteins and nuclease domains of DNA repair complexes AddAB, AdnAB, and RecBC. ACE: Argonaute-associated Cas4 family enzyme. Each representative protein is color-coded based on the family it belongs to. The protein length (in amino acids) is indicated in rings outside the tree.

(**B**) Maximum likelihood-based phylogenetic tree containing all prokaryotic Argonaute (pAgo) homologs identified in the RefSeq database containing at least scaffold level assemblies. Analysis performed as previously reported[13]. ACE: Argonaute-associated Cas4 family enzyme. pAgos associated with ACE proteins are indicated with closed red circles (ACE in same operon) and open red circles (ACE encoded elsewhere in operon).

High resolution PDFs of both figures are available at Mendeley Data .

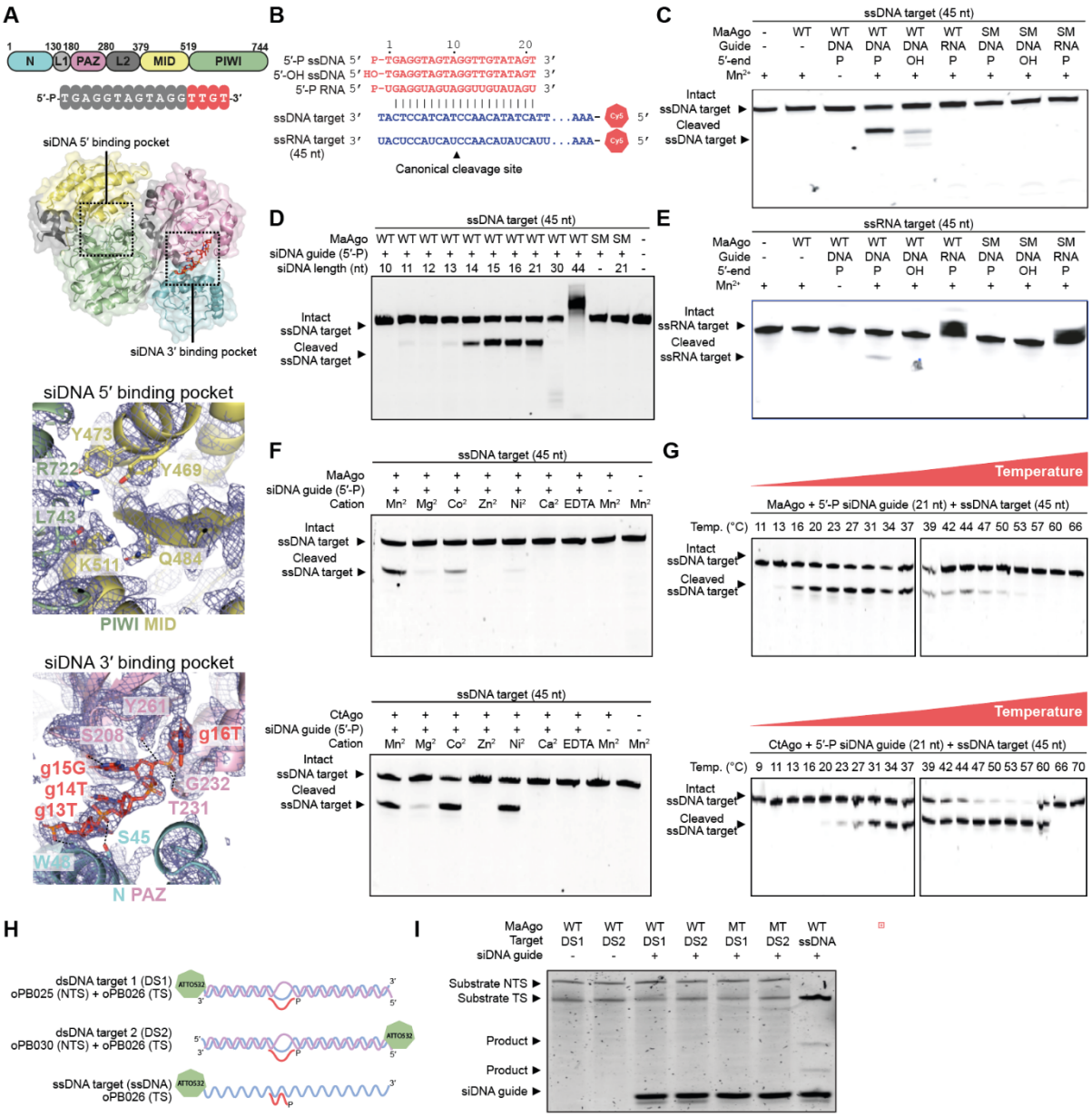

Figure S2. APACE pAgos mediate DNA-guided DNA cleavage. Related to Figure 2.

(**A**) Structural overview of the CtAgo-siDNA complex (upper panel) and electron density observed for the at canonical MID/PIWI siDNA 5′ end binding pocket (middle panel) and N/PAZ siDNA 3′ end binding pocket (lower panel). Electron density is displayed as blue mesh at σ level 1. (**B**) Sequences of guide and target oligonucleotides used in *in vitro* cleavage assays. (**C-G**) MaAgo (B-F) or CtAgo (E-F) was incubated with 21 nucleotide long ssDNA or RNA guides and Cy5-labelled ssDNA or RNA targets. Cy5-labelled cleavage products were resolved by denaturing gel (7M urea, 20% polyacrylamide) electrophoresis and visualized by fluorescence. SM: MaAgo catalytic single mutant with D537A substitution. (**C**) MaAgo can use siDNAs with a length ranging from 14 to 30 nucleotides to cleave ssDNA targets. 44 nucleotide long siDNAs bound to target ssDNAs do not resolve properly during electrophoresis. (**F**) MaAgo and CtAgo can use Mn^{2+}, Mg^{2+}, Co^{2+}, and Ni^{2+} for target strand cleavage. (**G**) MaAgo and CtAgo mediate siDNA-guided ssDNA cleavage at temperatures ranging from 16-50 °C or 23-60 °C, respectively. (**H**) Schematic overview of guides and targets used in panel (I). (**I**) MaAgo does not cleave dsDNA. TS: Target strand. NTS: Non-target strand. In all assays, MaAgo (C-H) or CtAgo (F-G) were incubated with guide and target at final concentrations of 0.4 μM:0.4 μM:0.1 μM (CtAgo:guide:target) in presence of 5 mM $MnCl_2$ or other divalent metals (E). After 1h of incubation, cleavage products were resolved by denaturing gel (7M urea, 20% polyacrylamide) electrophoresis and visualized using Cy5 fluorescence (ssDNA/ssRNA) or SYBR Gold staining (dsDNA).

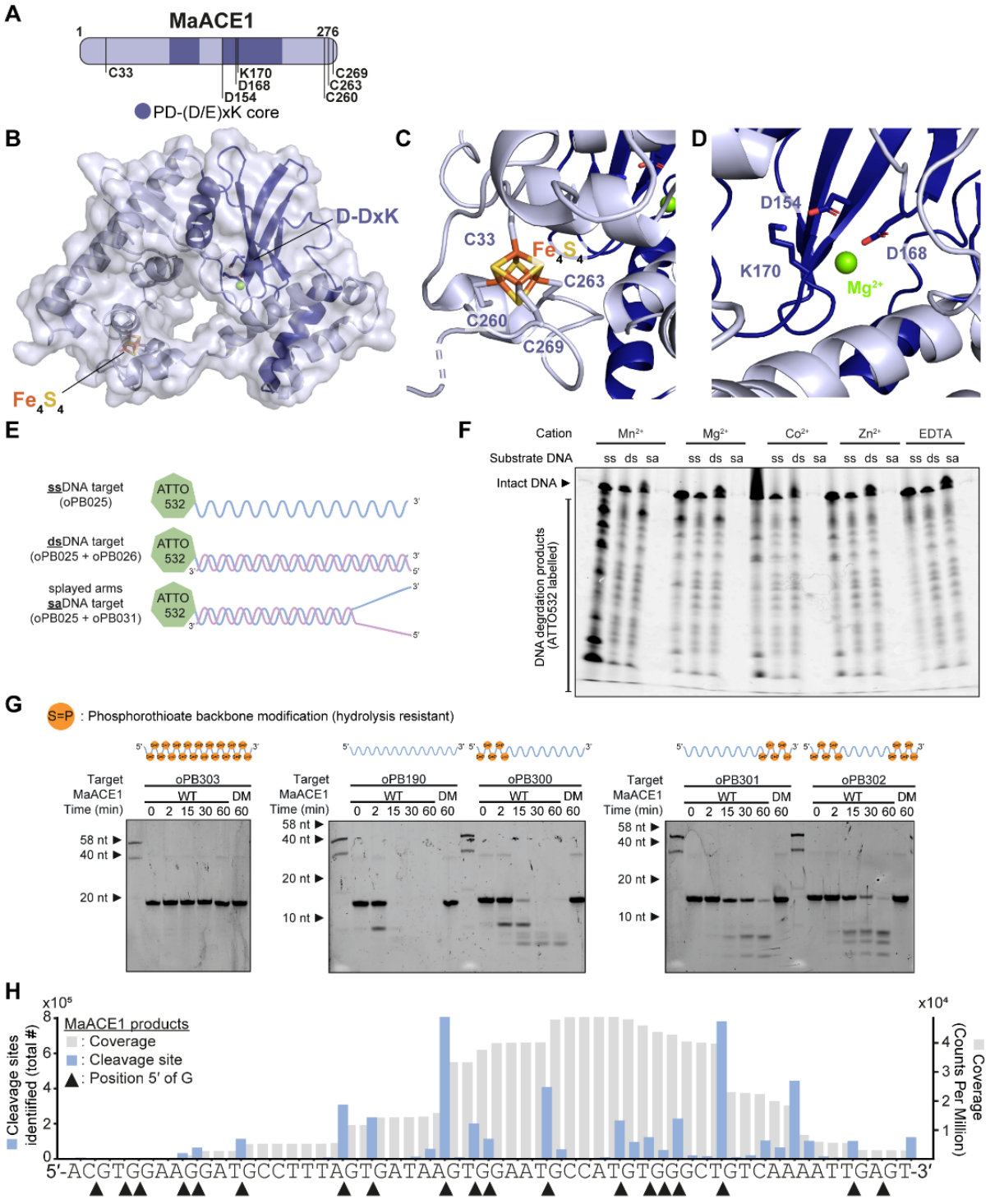

Figure S3. MaACE1 is a ssDNA endonuclease. Related to Figure 3.

(**A**) Schematic diagram of MaACE1. The structural segments corresponding to the core of PD-(D/E)xK family proteins is colored dark purple, and the remainder of MaACE1 light purple.

(**B**) Cartoon representation of the overall structure of the MaACE1, resolved at 2.07 Å resolution, colored according to the scheme in panel A.

(**C**) Close-up view of the MaACE1 catalytic triad, with Mg^{2+} ion bound (green sphere).

(**D**) Close-up view of the four cysteines that coordinate the Fe_4S_4 cluster.

(**E**) Schematic representation of single-stranded (ss), splayed-arm (sa), and double-stranded (ds)DNA substrates.

(**F**) MaACE1 cleaves ssDNA or unwound dsDNA. MaACE1 was incubated with ATTO532-labelled DNA substrates at final concentrations of 0.1 μM:0.4 μM (MaACE1:substrate) in presence of 5 mM of various divalent cations. After 10 min of incubation cleavage products were resolved by denaturing gel (7M urea, 20% polyacrylamide) electrophoresis and visualized by ATTO532 fluorescence.

(**G**) MaACE1 cleaves ssDNA as an endonuclease. MaACE1 was incubated with different ssDNA substrates with hydrolysis-resistant phosphorothioate modifications (indicated by yellow spheres with S=P) at final concentrations of 0.1 μM:0.4 μM (MaACE1:substrate) in presence of 5 mM Mg^{2+}. Cleavage products were resolved by denaturing gel (7M urea, 20% polyacrylamide) electrophoresis and visualized by SYBR gold staining. DM: MaACE1 catalytic mutant with D154A and D168A substitutions.

(**H**) MaACE1 preferentially cleaves ssDNA substrates upstream of 5′ end of guanine nucleotides. Cleavage sites were identified by next generation sequencing of cleavage products. Grey bars indicate read coverage for each nucleotide, blue bars indicate MaACE1 cleavage sites mapped on the ssDNA substrate.

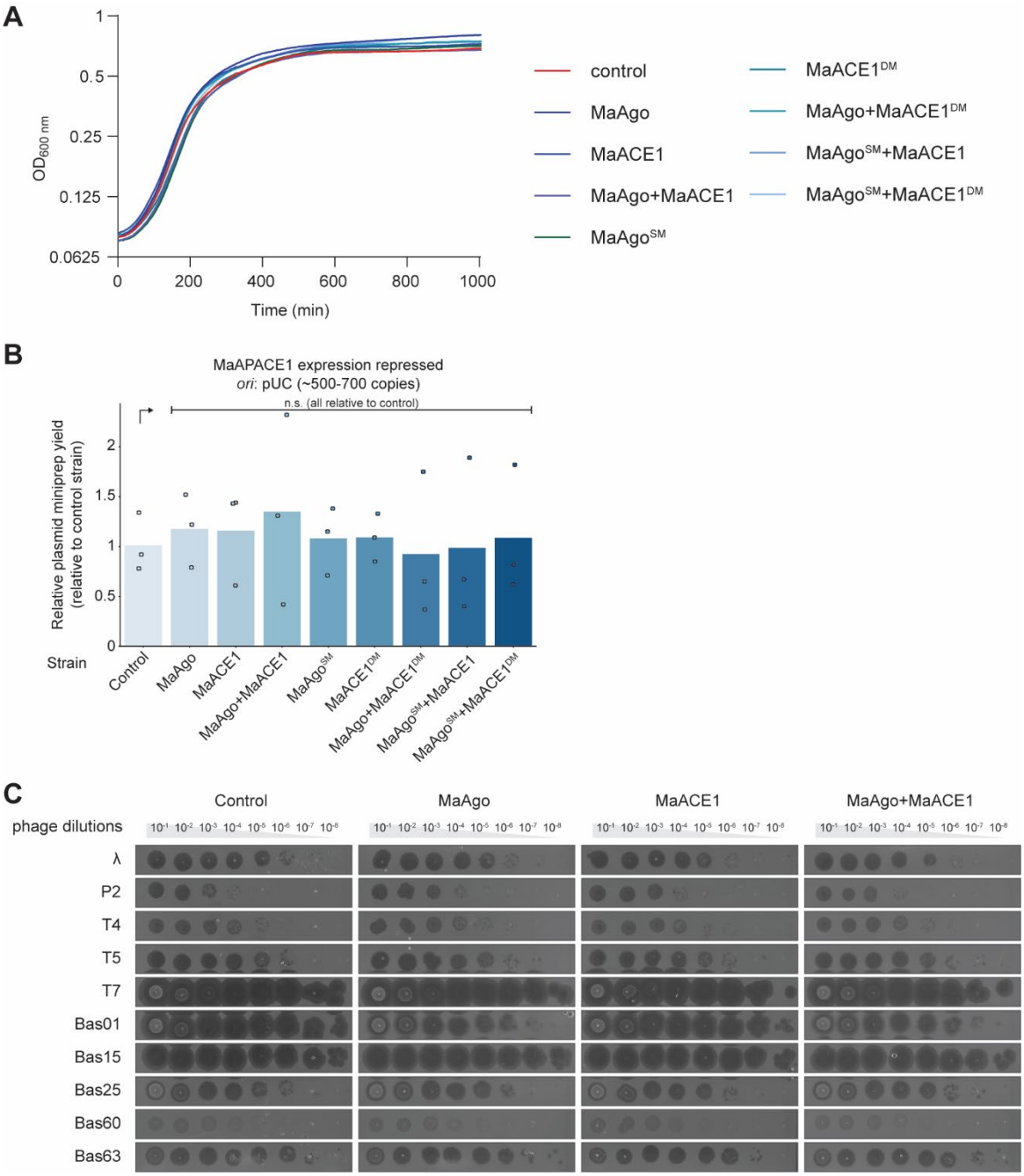

Figure S4. APACE1 protects against bacteriophage Bas01 infection. Related to Figure 6.

(**A**) APACE1 expression does not influence *E. coli* growth. Growth experiment of different *E. coli* BW25113 strains in which APACE1 proteins or mutants thereof are expressed in absence of bacteriophage infection. Lines represent averages of three biological replicates.

(**B**) pUC pBK157 plasmid yield after purification from *E. coli* BW25113 mutant strains encoding CAT and MaAgo, MaACE1, both MaAgo and MaACE1, and/or mutants thereof under repression conditions (in presence of glucose). Plasmid concentration was normalized against the plasmid content in the control strain. Experiments were performed in biological triplicates, data points indicate single experiments.

Statistical significance (p) was calculated using the student's t-test. n.s.=not significant; *=p<0.05; **=p<0.005; ***=p<0.0005.

(**C**) APACE1 protects against bacteriophage Bas01. Bacteriophage drop test assay on double-layered LB agar plates supplemented with 0.2% arabinose to induce the APACE system. *E. coli* BW25113 strains were challenged with 10-fold serial dilutions of various bacteriophages. Experiments were performed in biological triplicates – representative results of one experiment shown here.