Expanded View Figures

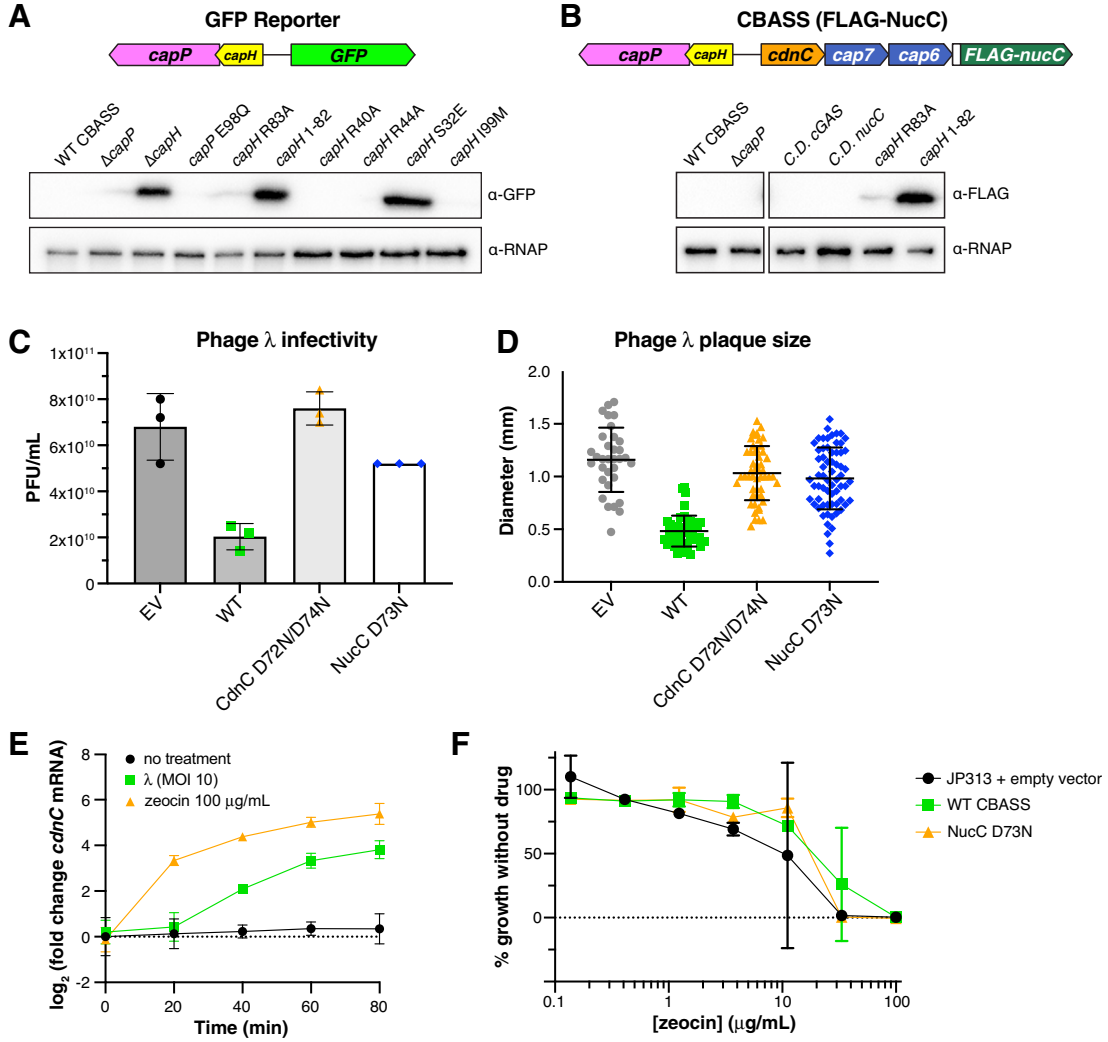

Figure EV1. CBASS expression reporter systems.

A Full Western blot for GFP reporter assay. α-RNAP, anti-RNA Polymerase loading control.

B Full Western blot for FLAG-NucC expression reporter assay. α-RNAP, anti-RNA Polymerase loading control.

C Quantitative plaque assay showing infectivity of λ cI⁻ against cells containing no CBASS system (EV, empty vector), the wild-type *E. coli* MS115-1 CBASS system (WT), or mutant systems with catalytic-dead CdnC (D72N/D74N) or NucC (D73N). Data are shown as average and standard deviation of plaque forming units per ml of purified phage (PFU/ml), from triplicate experiments. For NucC D73N, all three plates showed equal plaques (52 plaques at the tested dilution).

D Size of phage plaques for λ cI⁻ infecting cells containing no CBASS system (EV, empty vector; $n = 33$), the wild-type *E. coli* MS115-1 CBASS system (WT; $n = 46$), or mutant systems with catalytic-dead CdnC (D72N/D74N; $n = 51$) or NucC (D73N; $n = 65$). Data are shown as average and standard deviation of all plaques counted in panel (C).

E qRT–PCR for *cdnC* using JP313 cells containing the *E. coli* MS115-1 CBASS operon on a plasmid under exponential growth conditions (black circles), after phage λ infection at MOI 10 (green squares), or after addition of zeocin at 100 μg/ml (orange triangles). Error bars indicate standard deviation from three technical replicates each of three biological replicates (nine total measurements per condition).

F Minimum inhibitory concentration (MIC) analysis of JP313 cells with either empty vector (black circles), *E. coli* MS115-1 CBASS (green squares), or a mutant CBASS system with catalytic-dead NucC (D73N; orange triangles). Data shown are the average and standard deviation of three biological replicates (see Materials and Methods).

Source data are available online for this figure.

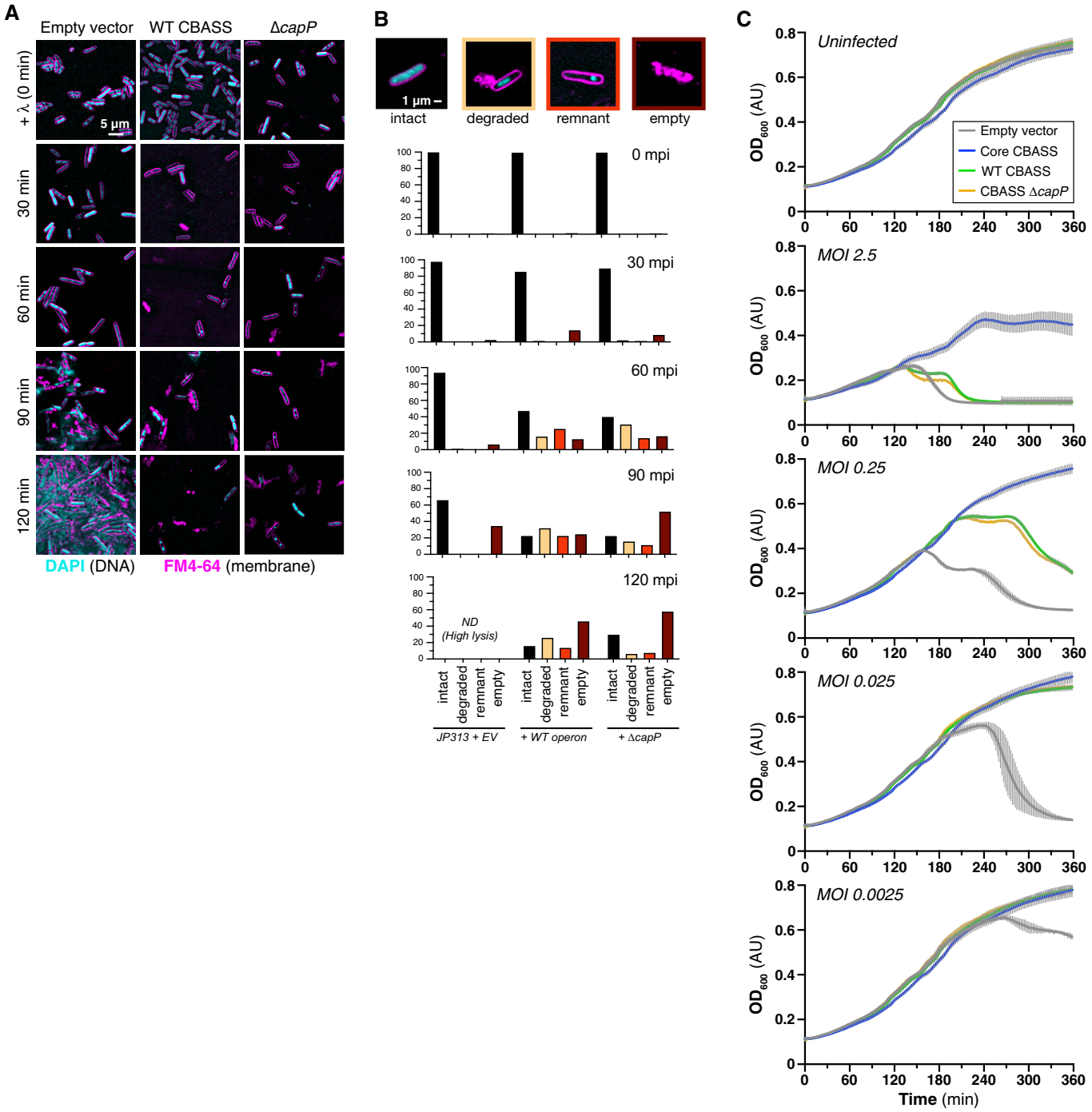

Figure EV2. Phage infection of cells with *E. coli* MS115-1 CBASS.

A Live-cell fluorescence microscopy of λ cI⁻ infecting cells containing no CBASS system (EV, empty vector), the wild-type *E. coli* MS115-1 CBASS system (WT), or a mutant system lacking *capP* (Δ*capP*). DNA (DAPI) is colored cyan, and cell membranes (FM4-64) are colored magenta.

B Quantification of fluorescence microscopy of λ cI⁻ infecting cells containing no CBASS system (EV, empty vector), the wild-type *E. coli* MS115-1 CBASS system (WT), or a mutant system lacking *capP* (Δ*capP*). Cells were manually quantified and assigned categories based on examples shown at top ($n \geq 30$ cells for each strain and time point).

C Growth curves of JP313 cells containing plasmids with no insert (Empty vector; gray), the core four CBASS genes under a lac-inducible promoter (Core CBASS; blue; Lau *et al*, 2020), the six-gene CBASS system (WT CBASS; green), or the six-gene CBASS system with capP deleted (CBASS Δ*capP*; orange). All strains grew comparably when not infected with phage (Uninfected, top) and showed differing levels of protection when infected with phage λ at different multiplicity of infection (MOI). Data shown are the average and standard deviation of three independent measurements.

Source data are available online for this figure.

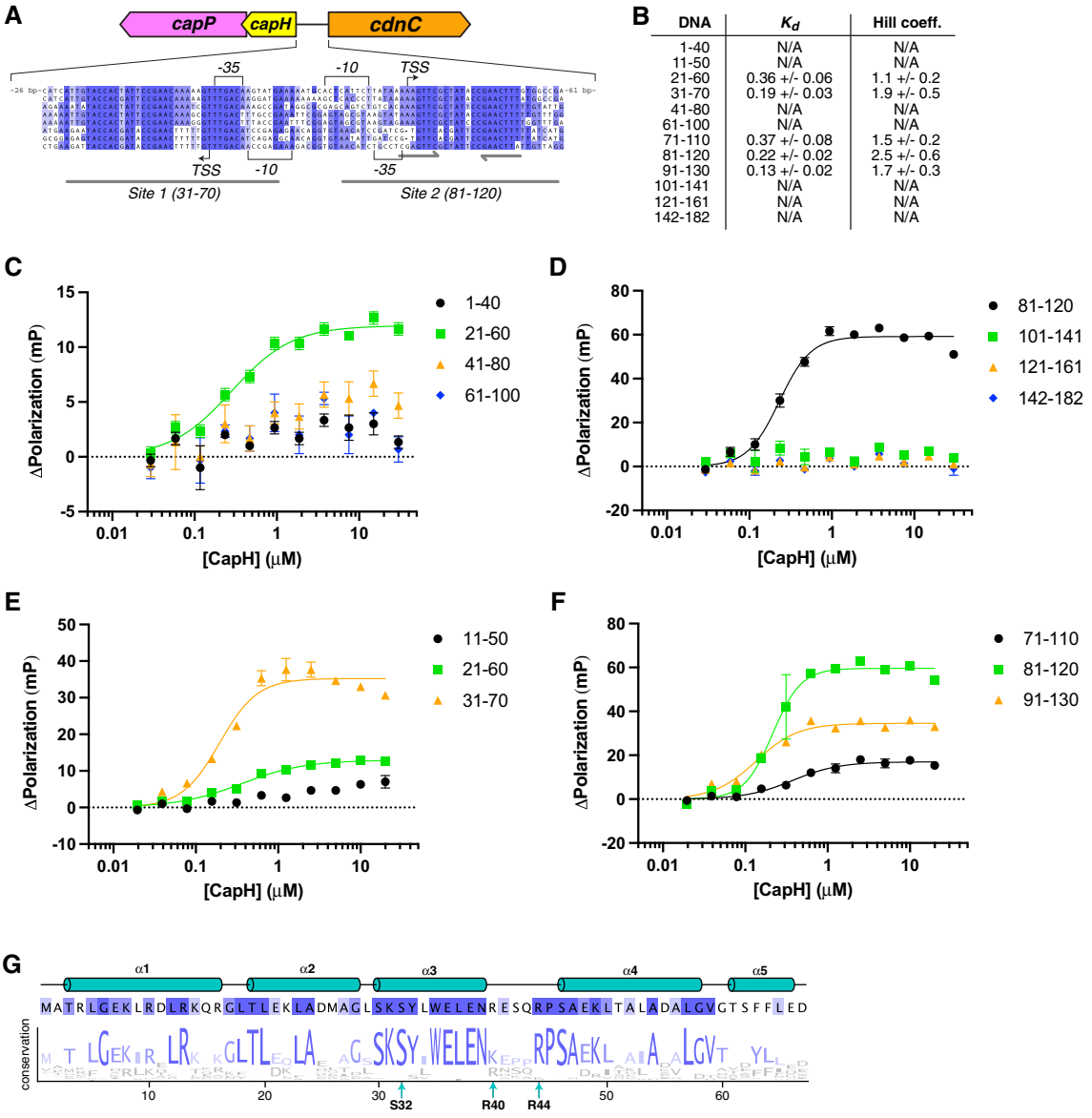

Figure EV3. CapH binds MS115-1 CBASS intergenic region.

A Sequence alignment of CBASS promoter regions in *E. coli* MS115-1 (NCBI RefSeq GG771785.1), *Cronobacter sakazakii* strain cro3915C2 (NZ_NRJY01000012.1), *Pseudomonas stutzeri* strain KC NODE_1_length_951488_cov_16.453 (NZ_POUN01000001.1), *Pseudomonas* sp. RIT 412 RIT412_S3_7 (NZ_QBJA02000007.1), *Pseudomonas* sp. MF4836 (NZ_MVOL01000002.1), *Burkholderia pseudomallei* strain MSHR4301 (NZ_LXCN01000015.1), *Ralstonia insidiosa* strain WCHRI065162 (NZ_PKPC01000011.1), *Thauera* sp. K11 plasmid pTX1 (NZ_CP023440.1). Promoter sequences (−35, −10, and TSS) were predicted by the BPROM server (Solovyev & Salamov, 2011). Identified CapH-binding Sites 1 and 2 are denoted by gray underlines, and the palindrome within Site 2 is denoted by gray arrows.

B DNA binding affinity (K_d) and cooperativity (Hill coefficient) from fluorescence polarization measurements of CapH binding overlapping 40–41 bp DNAs spanning the *E. coli* MS115-1 CBASS promoter region (Fig 2A).

C–F Fluorescence polarization assays showing binding of *E. coli* MS115-1 CapH (His_6-MBP tagged) to 40–41 bp DNAs spanning the *E. coli* MS115-1 CBASS promoter region. Fit K_d and Hill coefficient for each DNA is shown in panel (B). Error bars indicate standard deviation from three technical replicates.

G Schematic of the CapH NTD with sequence logo indicating the conservation of each residue in an alignment of 56 unique CBASS-associated CapH proteins.

Source data are available online for this figure.

Figure EV4. CBASS-associated CapP contains an internal cysteine switch.

A Operon schematic of the *Thauera* sp. K11 CBASS system, compared to the *E. coli* MS115-1 system, with sequence identity between the two systems' CapH and CapP proteins indicated.

B Structure of *Thauera* sp. K11 CapP, with close-up of its Zn^{2+} metallopeptidase domain (pink) with internal cysteine switch loop (blue) and cysteine switch residue (C113).

C Structure of human matrix metalloprotease MMP9 (PDB IF 1L6J; Elkins *et al*, 2002), with close-up of its Zn^{2+} metallopeptidase domain (pink) and N-terminal cysteine switch domain (blue) and cysteine switch residue (C99). The orientation of the Zn^{2+} metallopeptidase domains in panels (B) and (C) is identical.

D Evolutionary tree of 408 CBASS-associated CapP proteins, colored by the presence (black) or absence (blue) of the internal cysteine switch.

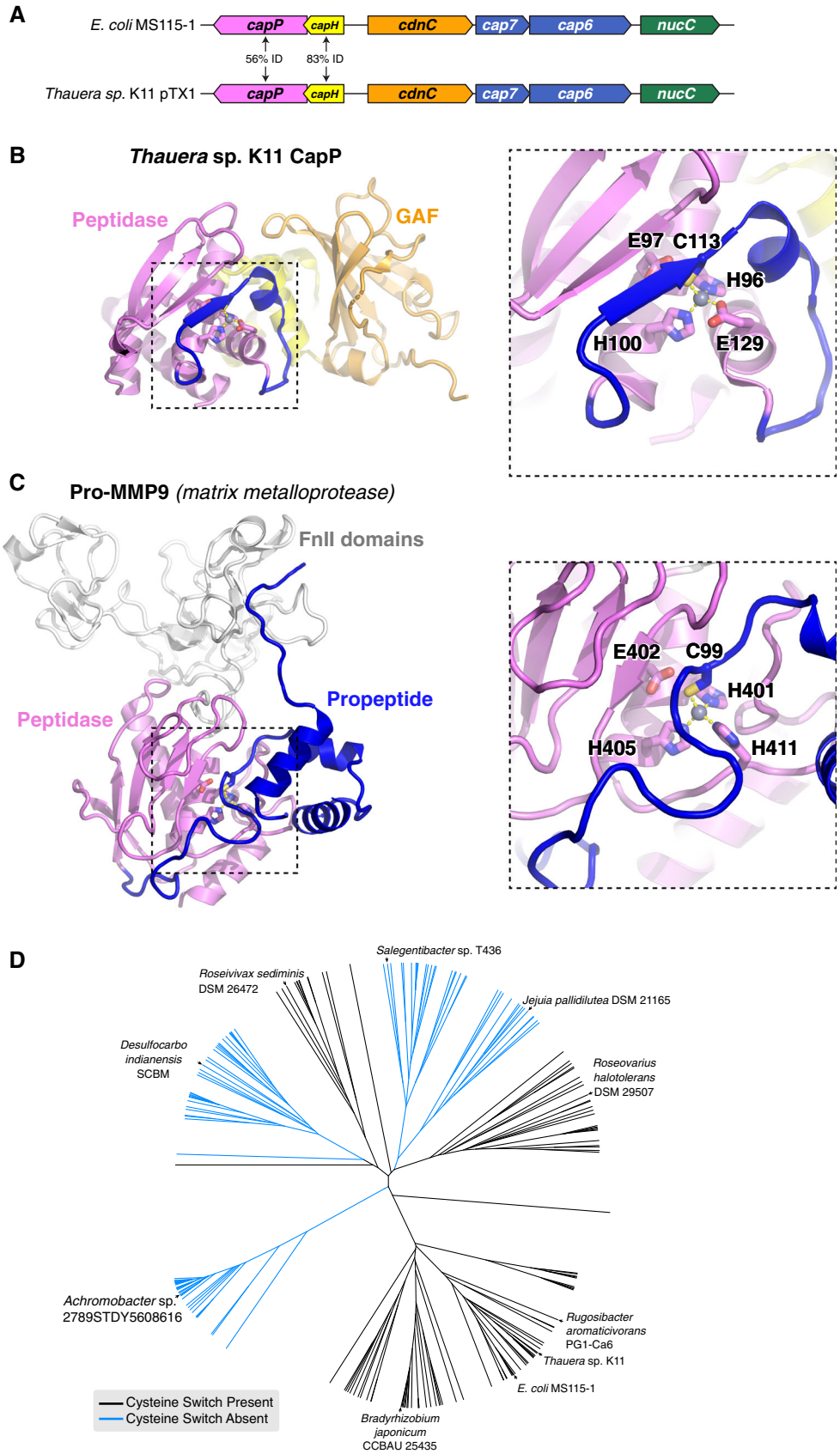

Figure EV4.

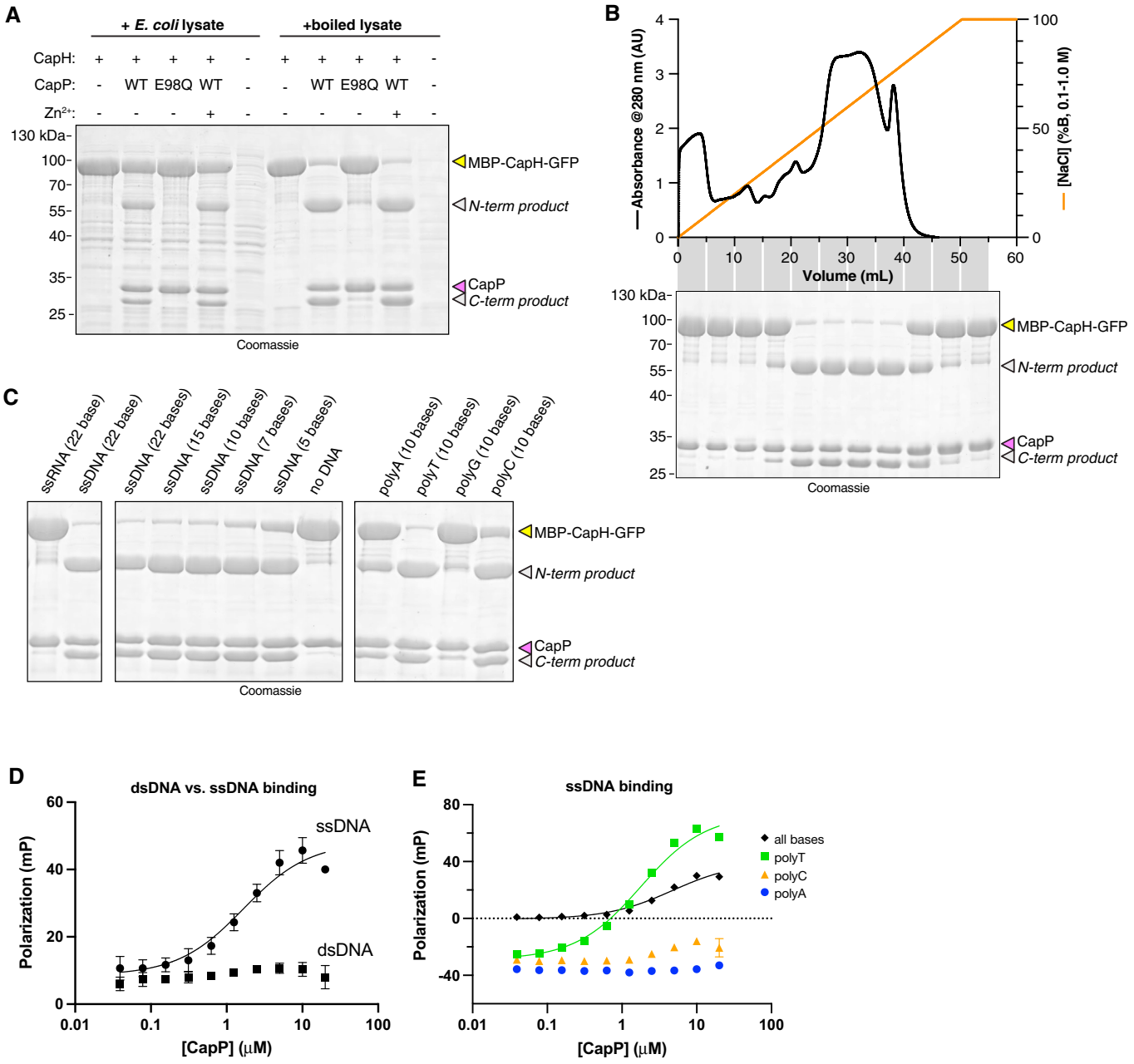

Figure EV5. DNA-mediated activation of CapP.

A *In vitro* cleavage assay with purified *E. coli* MS115-1 CapP (wild-type or catalytic-dead E98Q mutant), MBP-CapH-GFP, and *E. coli* cell lysate.

B *Top*: UV absorbance (280 nm) of boiled *E. coli* cell lysate separated by HiTrap Q column. Gradient (orange line) goes from 100 mM NaCl (0% B) to 1 M NaCl (100% B). *Bottom*: Coomassie-stained SDS–PAGE gel of cleavage assay with purified *E. coli* MS115-1 CapP, MBP-CapH-GFP, and combined fractions from HiTrap Q column. A similar assay with a Superdex 75 gel filtration column showed high activity across the entire separation range.

C *In vitro* cleavage assay with purified *E. coli* MS115-1 CapP, MBP-CapH-GFP, and the indicated nucleic acids.

D Fluorescence polarization DNA binding assay for *E. coli* MS115-1 CapP and either single-stranded DNA (circles and solid line; K_d = 1.7 ± 0.6 μM) or double-stranded DNA (squares; no binding detected). Error bars indicate standard deviation from three technical replicates.

E Fluorescence polarization DNA binding assay for *E. coli* MS115-1 CapP and single-stranded DNAs including a random sequence with all four bases (black diamonds; K_d = 4.7 ± 1.2 μM), poly-T (green squares; K_d = 1.8 ± 0.3 μM), poly-C (orange triangles; no binding detected), or poly-A (blue circles; no binding detected). Error bars indicate standard deviation from three technical replicates.

Source data are available online for this figure.