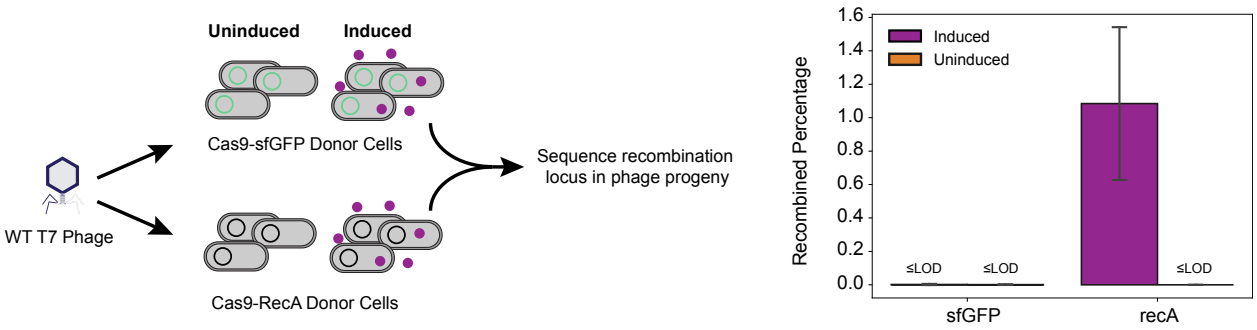
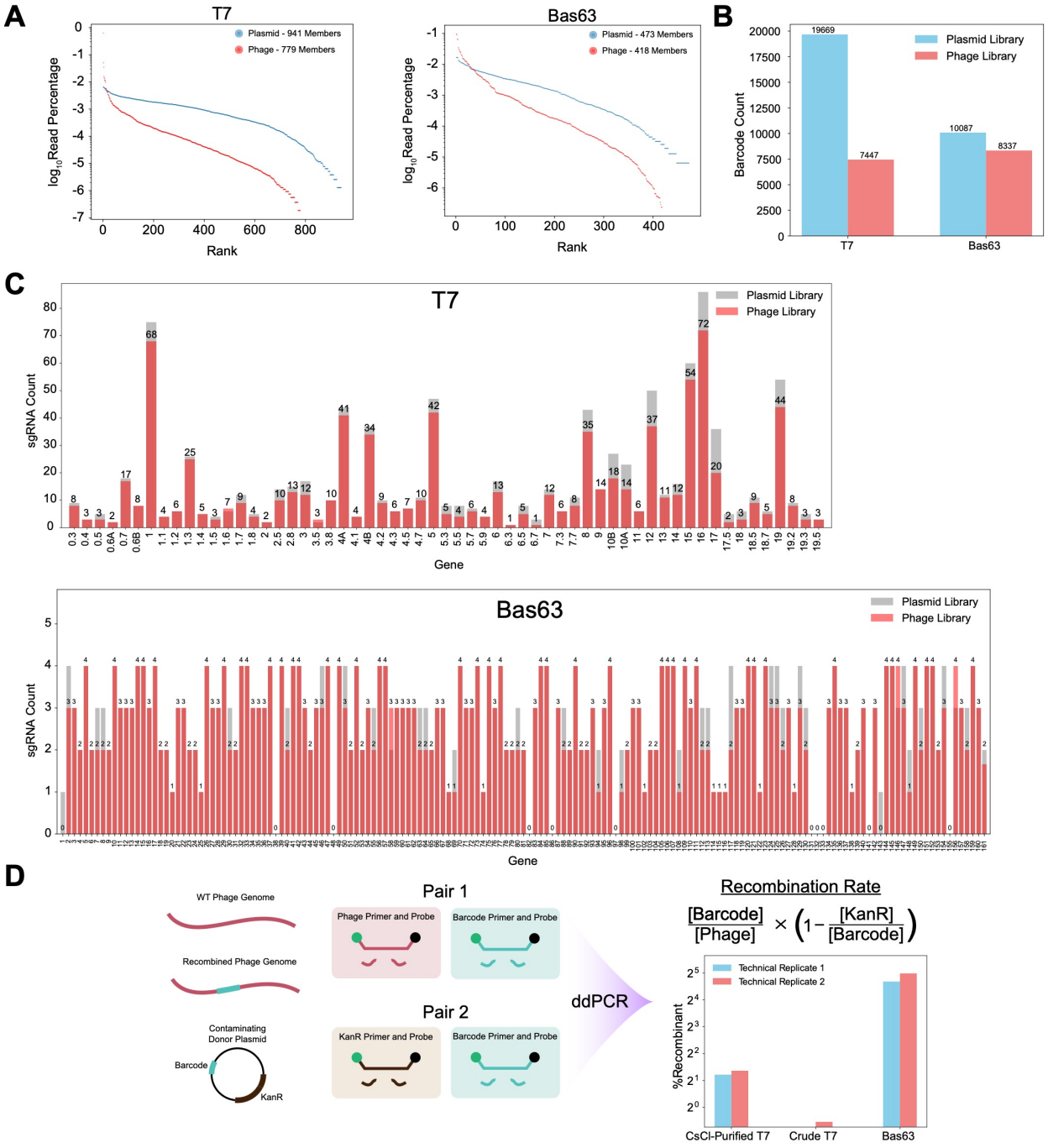
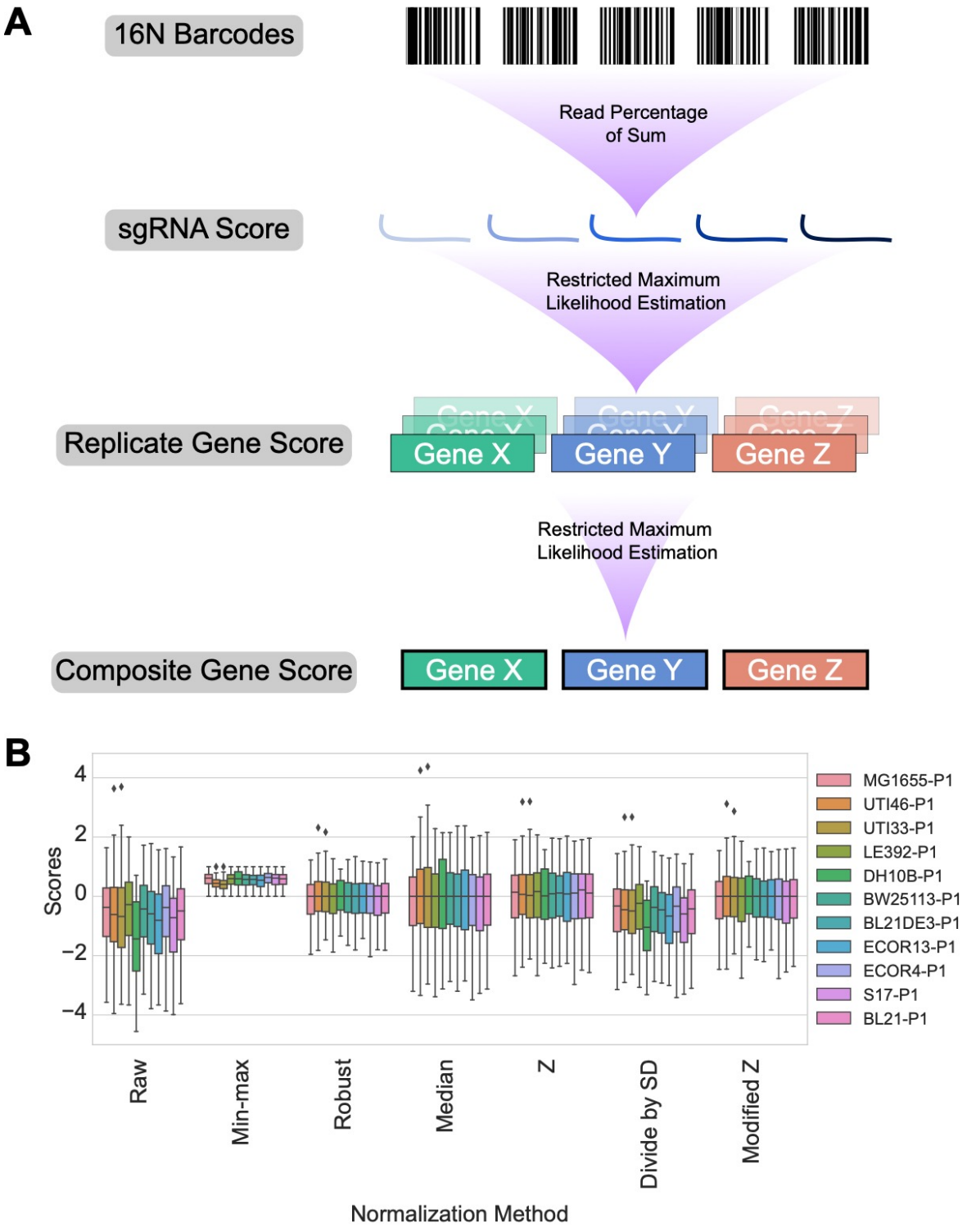


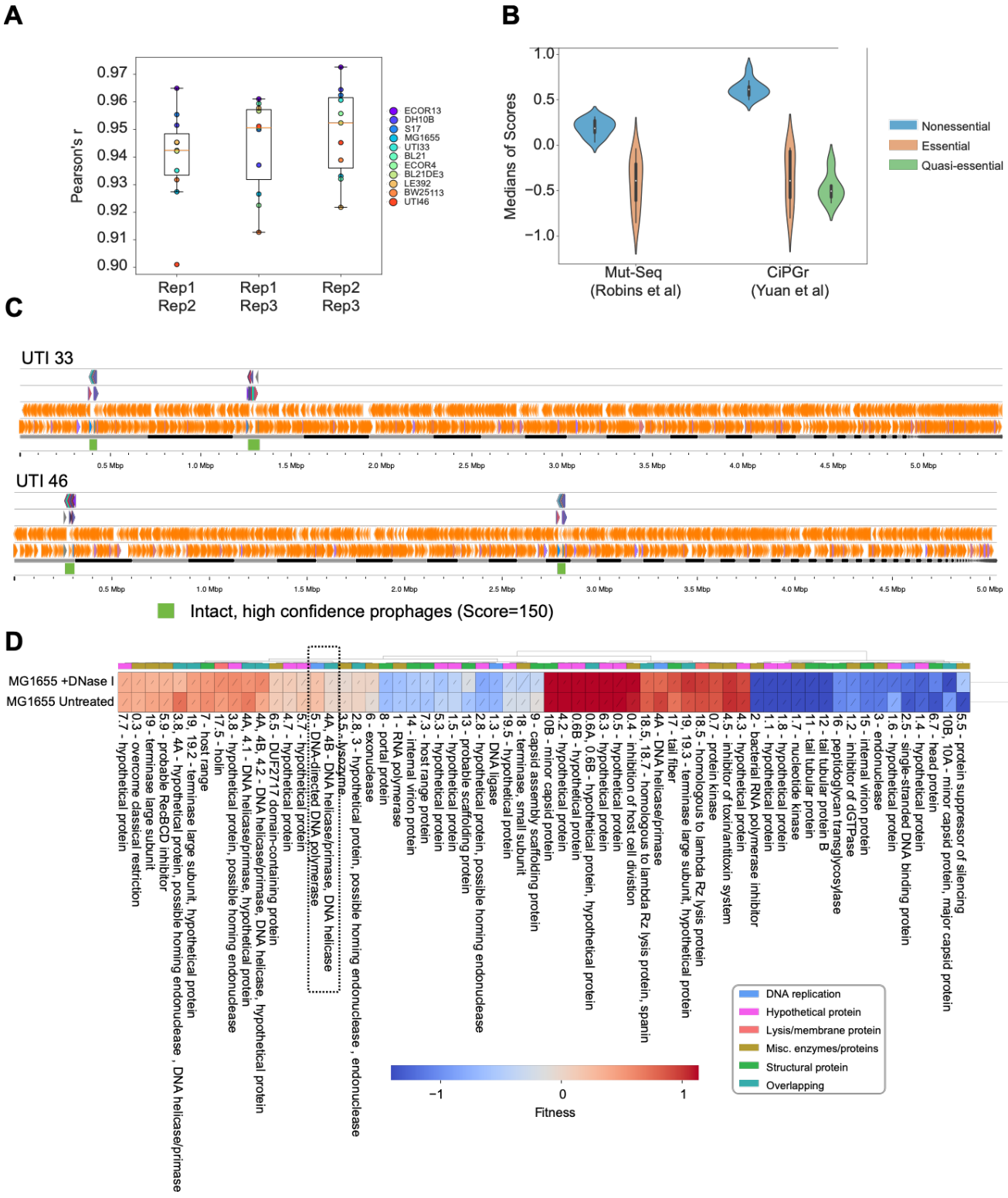
Supplementary Figure 1. Workflow for quantifying recombination efficiency with and without recA. Cells containing donor plasmids that would replace a 100bp fragment of g1.1 in T7 with a 100bp primer binding sequence-flanked barcode were co-transformed with a Cas9-sfGFP or Cas9-RecA plasmid. Cells were either uninduced or induced with 1% anhydrotetracycline. The cultures were infected by WT T7 phage and the resulting progeny phages were used as template for PCR amplification of the target locus for sequencing. Recombined percentages were calculated as percent of total reads that contain a consensus region found in the barcode insertion. Bars represent the average (n=3 biological replicates) recombined percentages. Error bars denote standard deviation. LOD is the limit of detection which is 1 read.



Supplementary Figure 2. Analysis of PhageMaP library quality for T7 and Bas63. (A) Rank-order curves for the T7 and Bas63 library members at the donor plasmid and phage library stages. **(B)** Total barcode counts for the T7 and Bas63 library members at the donor plasmid and phage library stages. **(C)** sgRNA representation for each gene of T7 and Bas63 at the donor plasmid and phage library stages. **(D)** Estimation of recombination rate of phage libraries with digital droplet PCR (ddPCR). Low %Recombinant for "Crude T7" samples indicate high contamination of donor plasmid DNA which is largely removed after cesium chloride (CsCl) ultracentrifugation.

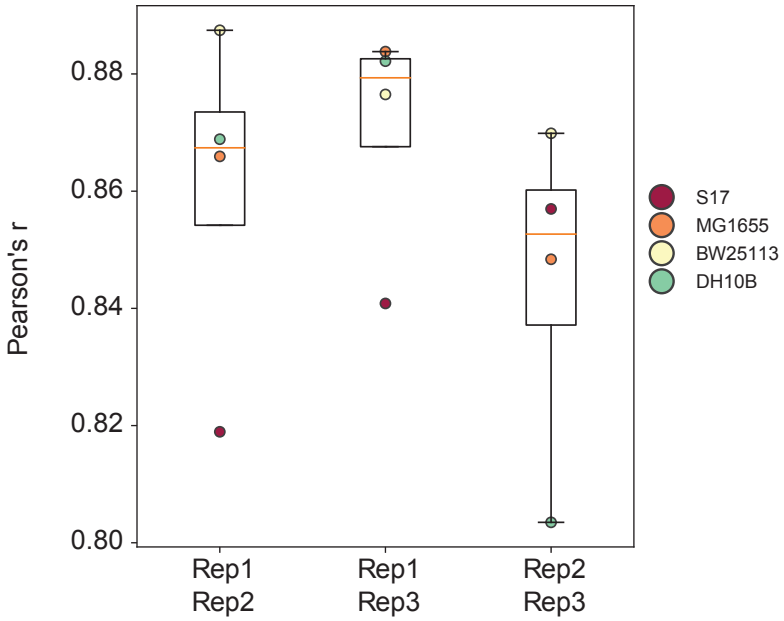


Supplementary Figure 3. PhageMaP scoring and analysis (A) Pipeline for calculating raw fitness scores from barcode counts. **(B)** Analysis of different methods for normalization. Boxplots represent the distribution of fitness scores after indicated normalization method.

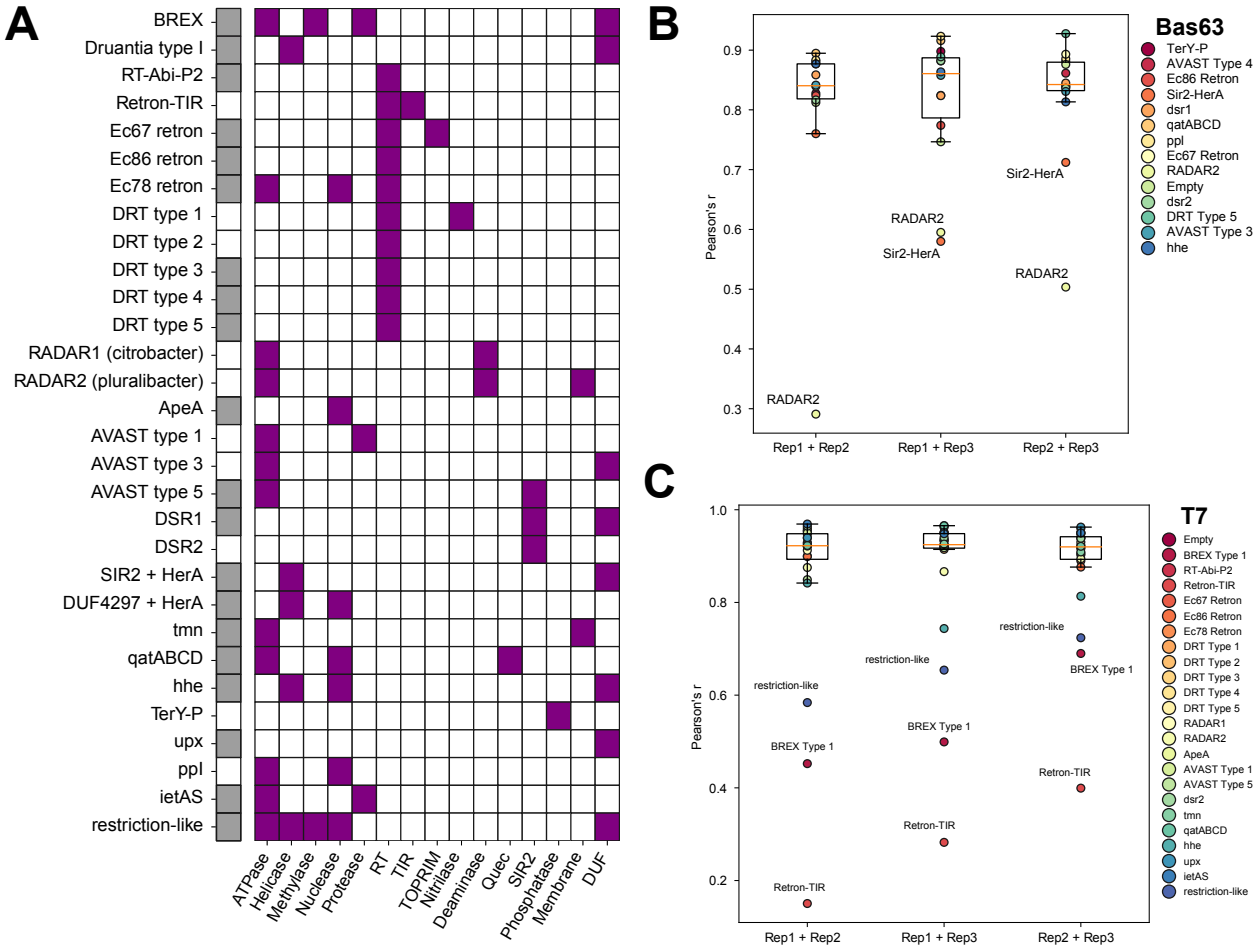


Supplementary Figure 4. Quality of T7 PhageMaP library screening on common labaoratory *E. coli* panel (A) Pair-wise Pearson's correlation between fitness scores of replicates in each of the 11 hosts. **(B)** Comparison of PhageMaP screen results with results from previous studies with T7. The classification of each gene from the indicated study was used to

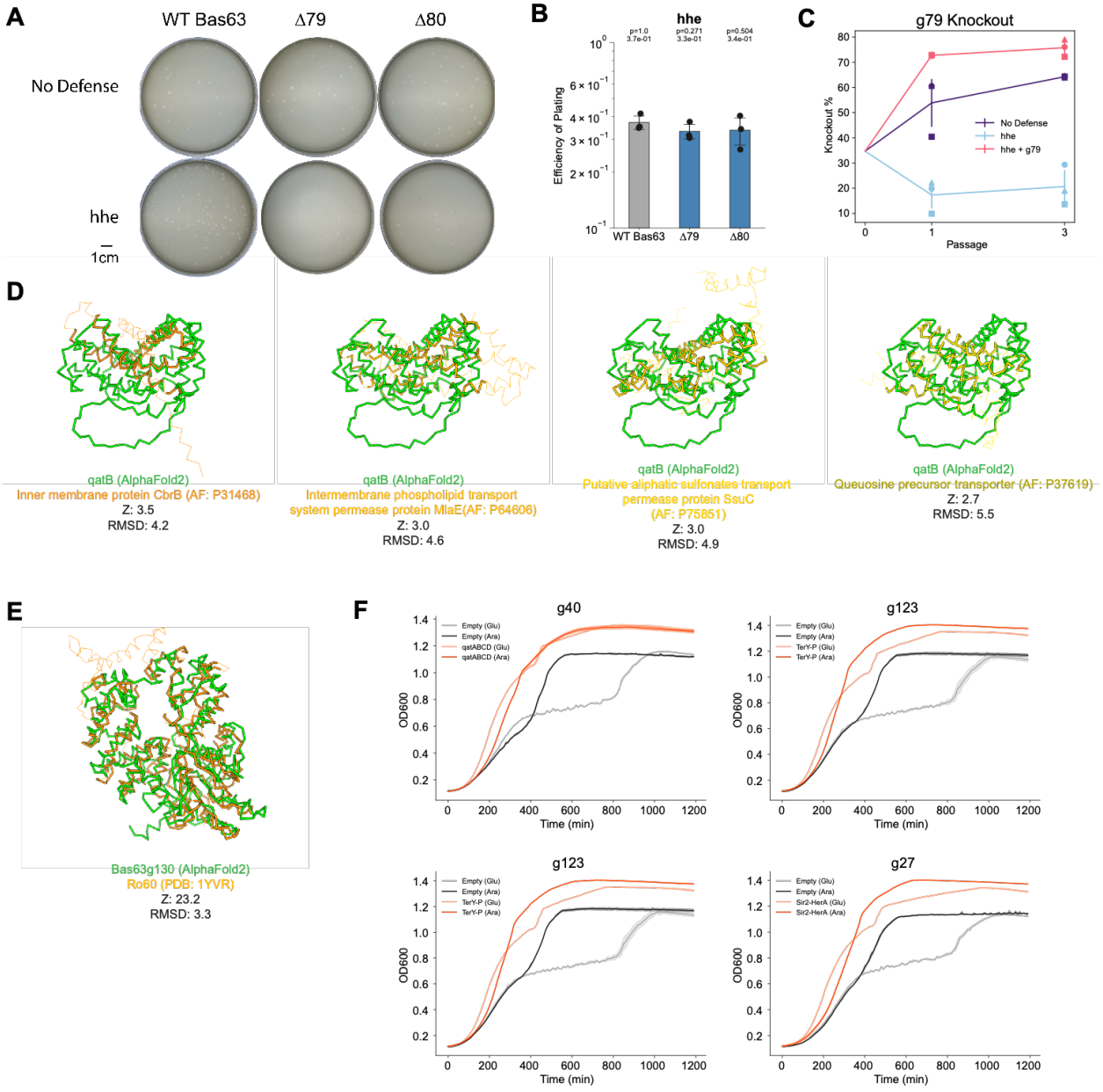
group genes. The median PhageMaP score for each group was then calculated for each of the 11 hosts. The violin plot represents the median values from those 11 hosts. **(C)** Genome tracks of UTI33 and UTI46 highlighting regions with intact, high confidence prophages (green). **(D)** Heatmap of T7 fitness scores after Z-normalization for DNase-treated or untreated MG1655 lysates. A 10^{th} percentile floor and 90^{th} percentile ceiling were imposed for the color scaling. The lines through each cell represent the standard error (SE) where a full line represents SE≥1. Clustering was performed using the Euclidean metric and Ward linkage method. Metadata describes general classification of each gene. Dotted box indicates genes which were hypothesized to have different scores after DNase treatment.



Supplementary Figure 5. Bas63 PhageMaP reproducibility. Pair-wise Pearson's correlation between fitness scores of replicates in the common laboratory *E. coli* panel.

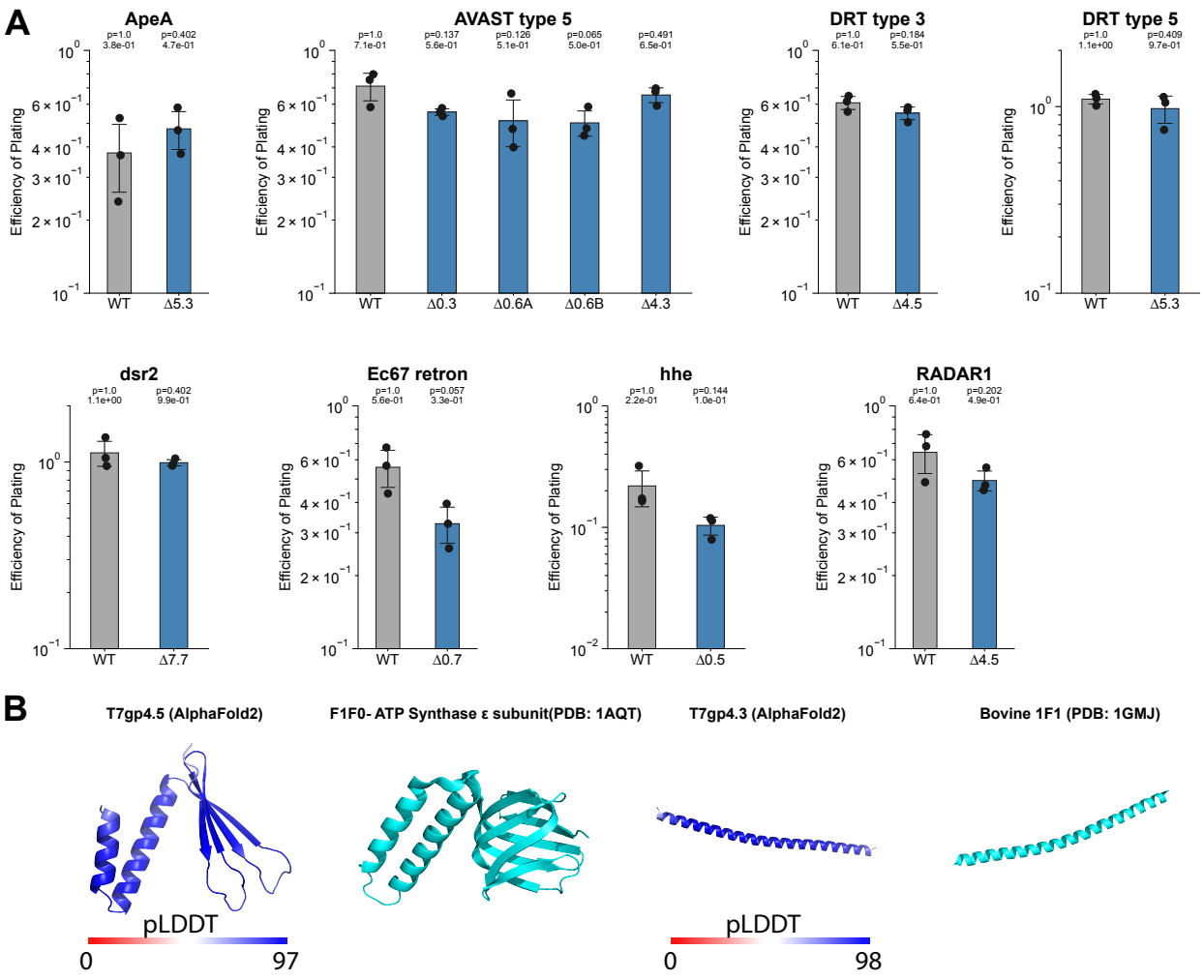


Supplementary Figure 6. PhageMaP screen using an anti-phage defense panel. (A) Enzymatic domains of each defense system in tested panel**.** Filled colored box indicate presence of enzymatic domain. Filled gray boxes on the left indicate system is of *E. coli* origin. (**B)** Pair-wise Pearson's correlation between fitness scores of replicates in the Bas63 screen. **(C)** Same as (B) but for T7.

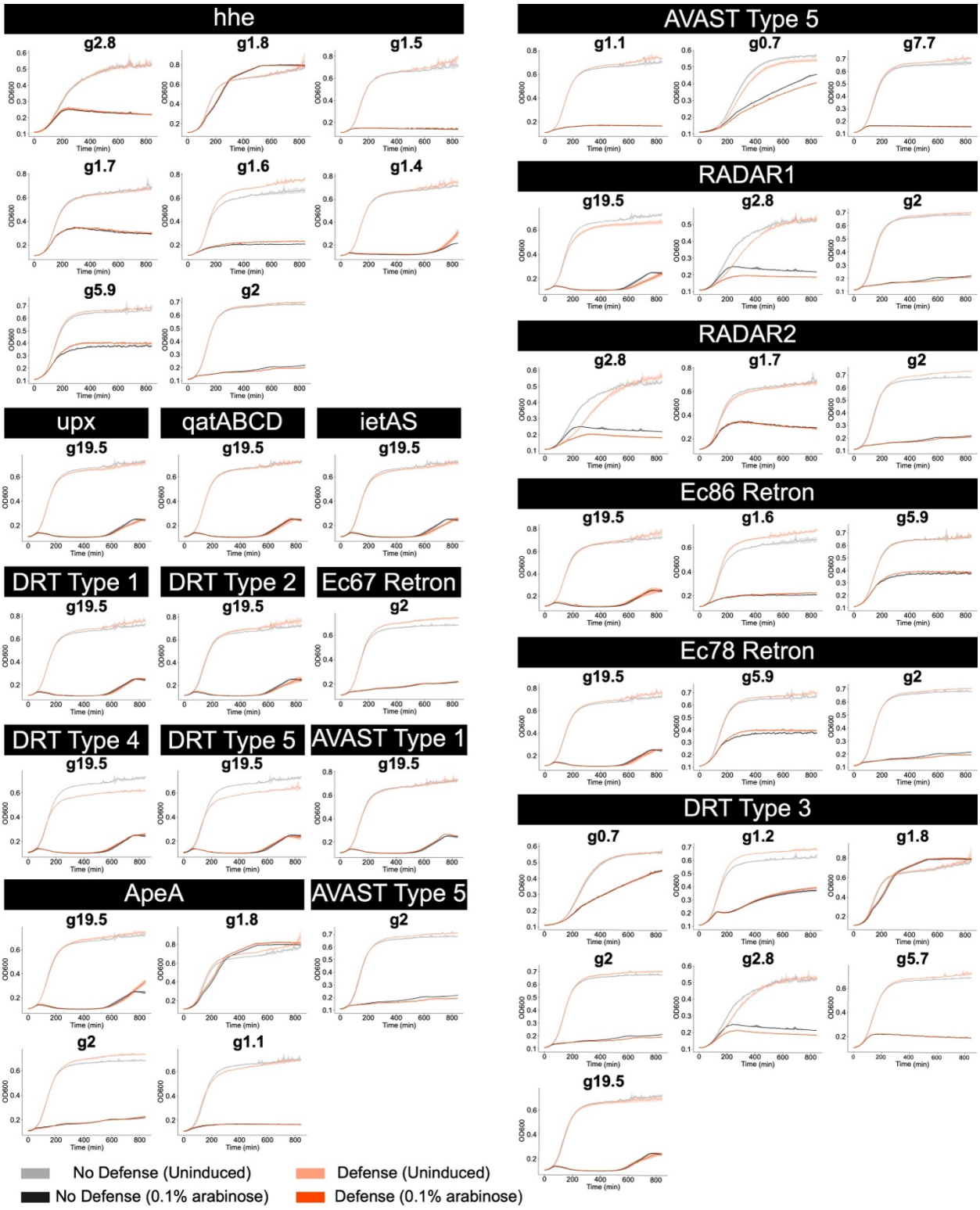


Supplementary Figure 7. Additional analysis for Bas63 anti-phage defense screen. (A) Plaque morphologies of knockouts grown on the No-defense and *hhe* strains. **(B)** Efficiency of plating (EOP) measurements for Bas63 knockouts with the *hhe* host. Average titers on the control strain were first determined (3 technical replicates). Each point on the plot represents a technical replicate for EOP using those calculated averages as the denominator for each titer measurement on the defense host. Bars represent the mean of three replicates, and error bars denote the standard deviation. Hypothesis testing was performed pairwise with each knockout and WT. P-values (Welch's t-test) and means are annotated above the bars. **(C)** Competition assay between barcoded WT Bas63 and Bas63Δ*g79* phages. Barcoded phages were mixed and passaged on the indicated hosts. Barcodes were sequenced and quantified to determine the ratio of Bas63Δ*g79* phages after each passage. **(D)** DALI structural comparisons of qatB with four membrane associated proteins. QatB (green) structure was predicted using

AlphaFold2 and compared to structures from the AlphaFold Database v2 (yellow/orange). Thick yellow/orange ribbons represent aligned regions of subjects. **(E)** DALI structural comparison of Bas63gp130 (green) with Ro60 (orange). Thick orange ribbons represent aligned region of Ro60. **(F)** Additional growth curves for identified Bas63 phage defense triggers. Growth curves for validation of Bas63 phage defense triggers for different systems. Cells containing either an empty vector or the indicated defense systems were transformed with an arabinose-inducible expression construct for the specified gene. Optical density measurements were acquired every 10 minutes for 1200 total minutes. Central line represents the average of 3 replicates and the shaded flanking regions represent the standard deviation.



Supplementary Figure 8. Additional analysis for counters identified from T7 anti-phage defense screen (A) Additional efficiency of plating (EOP) measurements for T7 knockouts under different phage defense systems. EOP measurements for gene knockouts (potential counters) in the context of indicated phage defense system. Average titers on the empty vector were first determined (3 technical replicates). Each point on the plot represents a technical replicate for EOP using those calculated averages as the denominator for each titer measurement on the defense host. Error bars represent standard deviation. Hypothesis testing was performed pairwise with each knockout and WT. P-values (Welch's t-test) and means are annotated above the bars. **(B)** Structural comparisons of T7gp4.5 and T7gp4.3 with the ε subunit of ATP Synthase and 1F1. T7gp4.5 and T7gp4.3 structures were predicted using AlphaFold2 and colored according to pLDDT confidence scoring. ε subunit of ATP Synthase (PDB: 1AQT) and bovine 1F1 (PDB: 1GMJ) structures were acquired from Protein Data Bank.



Supplementary Figure 9. Additional growth curves for expression of T7 phage genes under different phage defense systems. Growth curves for validation of T7 phage defense triggers for different systems. Cells containing either an empty vector or the indicated defense systems were transformed with an arabinose-inducible expression construct for the specified

gene. Optical density measurements were acquired every 10 minutes for 850 total minutes. Central line represents the average of 3 replicates and the shaded flanking regions represent the standard deviation.