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Supplementary Materials for

Unexpected evolutionary benefit to phages imparted by bacterial CRISPR-Cas9

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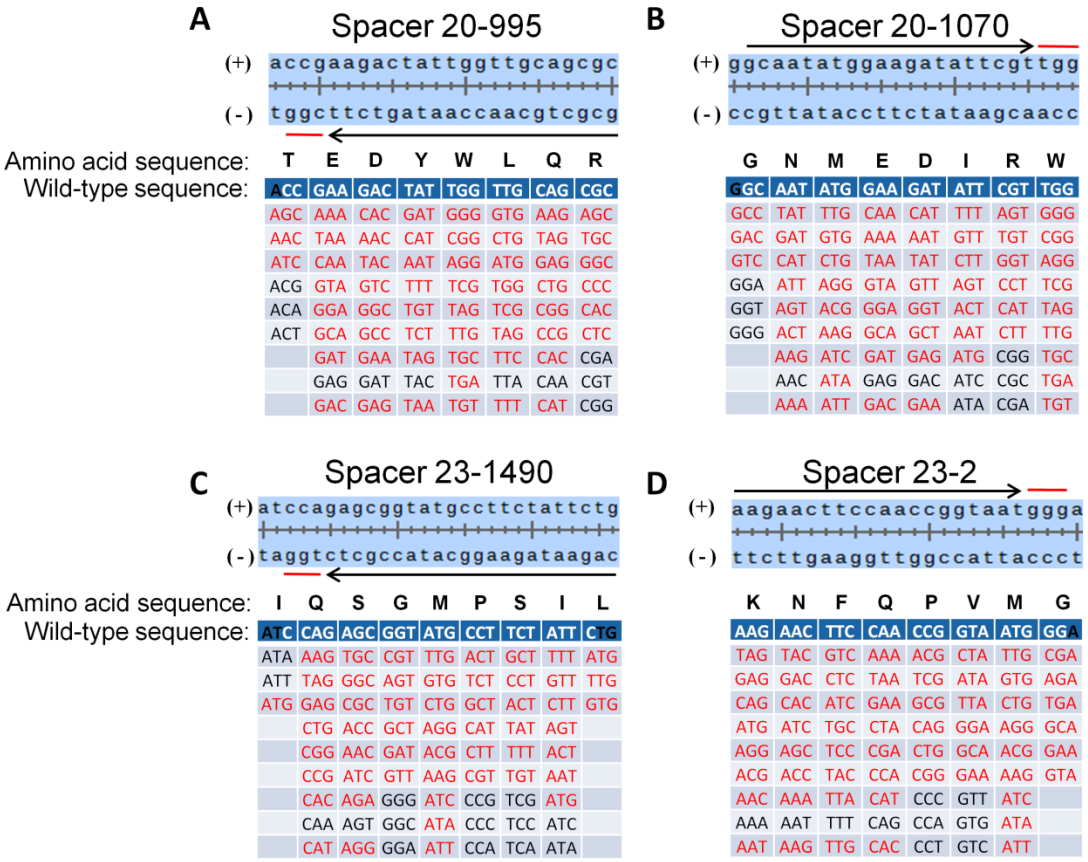

fig. S1. List of all possible single mutations in each spacer. The spacer regions were shown on top of each panel. The spacer sequences and PAMs are shown with arrows and red lines respectively. The possible single mutations of each codon of spacers 20-995 (**A**), 20-1070 (**B**), 23-1490 (**C**), and 23-2 (**D**) were shown in each table, where the wild-type sequences and corresponding amino acids were list on top. The silent mutations were shown in black color.

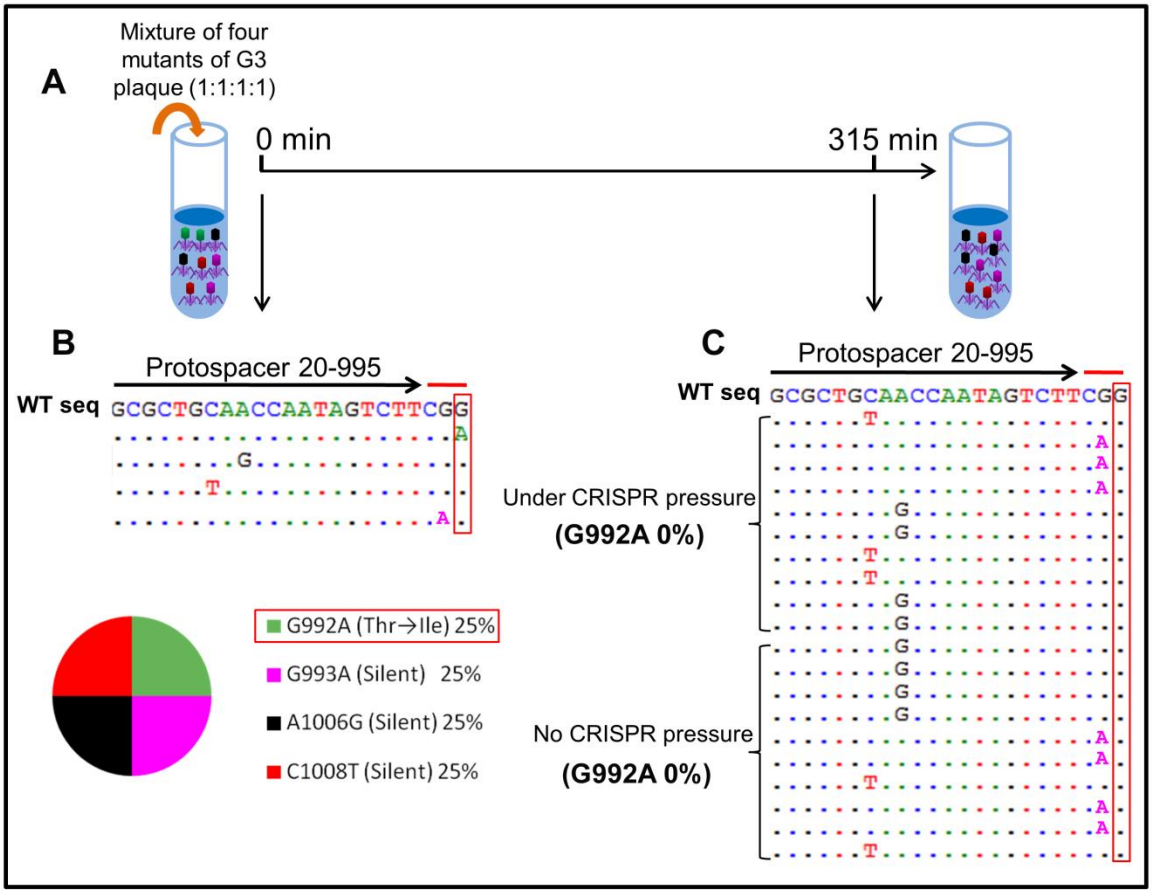

fig. S2. Relative fitness of the CEMs that escaped Cas9 cleavage of the protospacer 20-995 of the portal protein gene. Four phage mutants with nucleotide changes G991 to A (Thr to Ile), G992 to A (silent mutation), A1005 to G (silent mutation), and C1007 to T (silent mutation), were mixed in equal ratios and used to infect *E. coli* DH5α with or without the CRISPR-Cas9 plasmid (**A**, **B**). Three hundred and fifteen min after infection, the progeny phages were collected and plated on LB plates after serial dilution. Twenty single plaques were picked, PCR-amplified, and sequenced (**C**). The spacer sequence and PAM are marked with arrow and red line respectively. The percentages of each CEM in the starting sample and after 315 min evolution are shown in the pie charts (B, C).

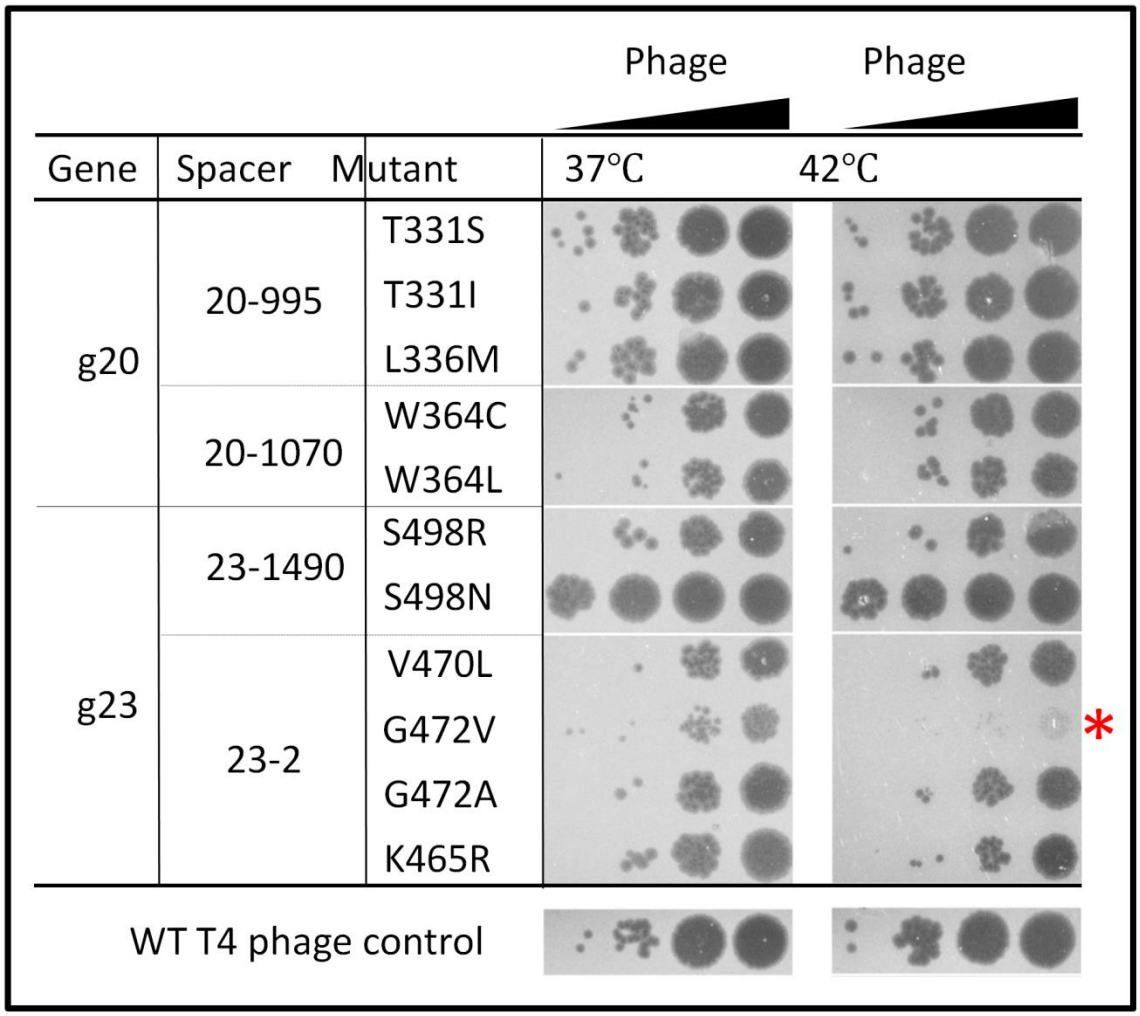

fig. S3. Temperature sensitivity of the CEMs containing amino acid changes. The mutant phages were spotted on *E. coli* DH5α lawn for lytic growth at 37°C or 42°C. The amino acid changes and their location in genes 20 or 23 are shown. * indicates the heat-sensitive phenotype of the G472V CEM in *g23*.