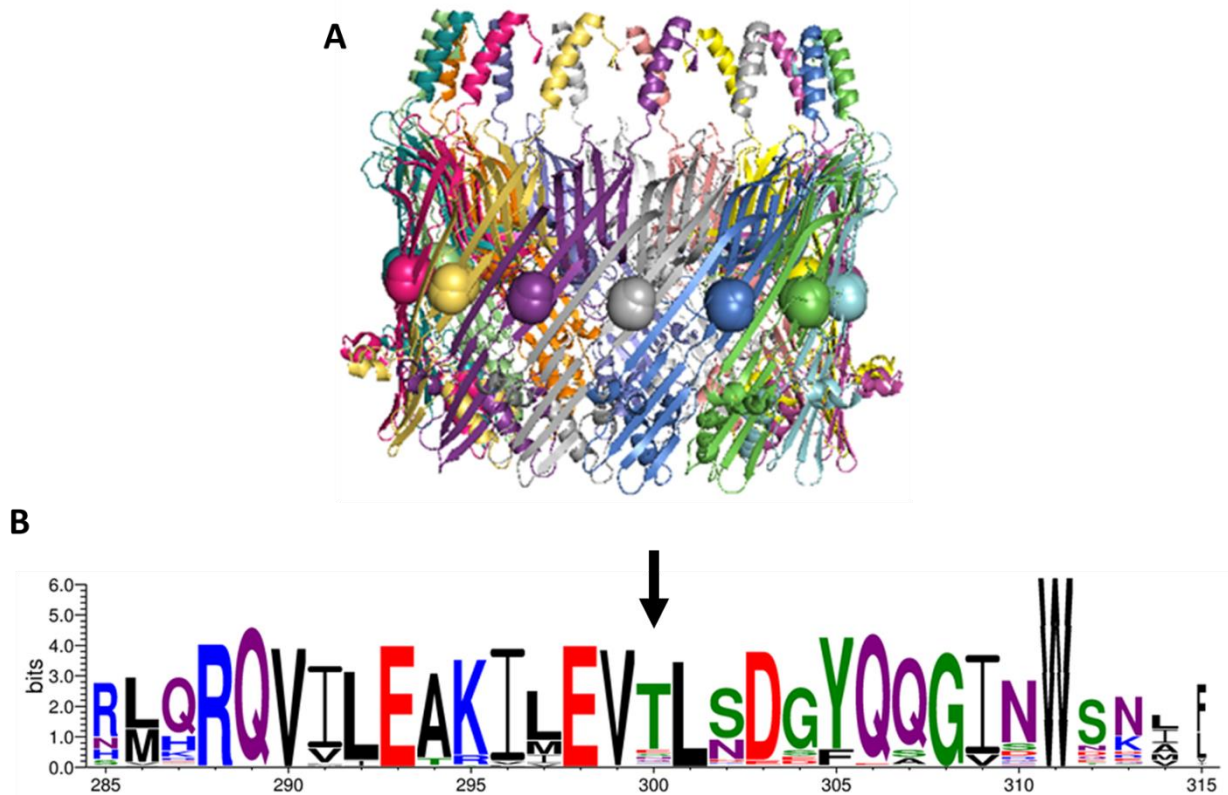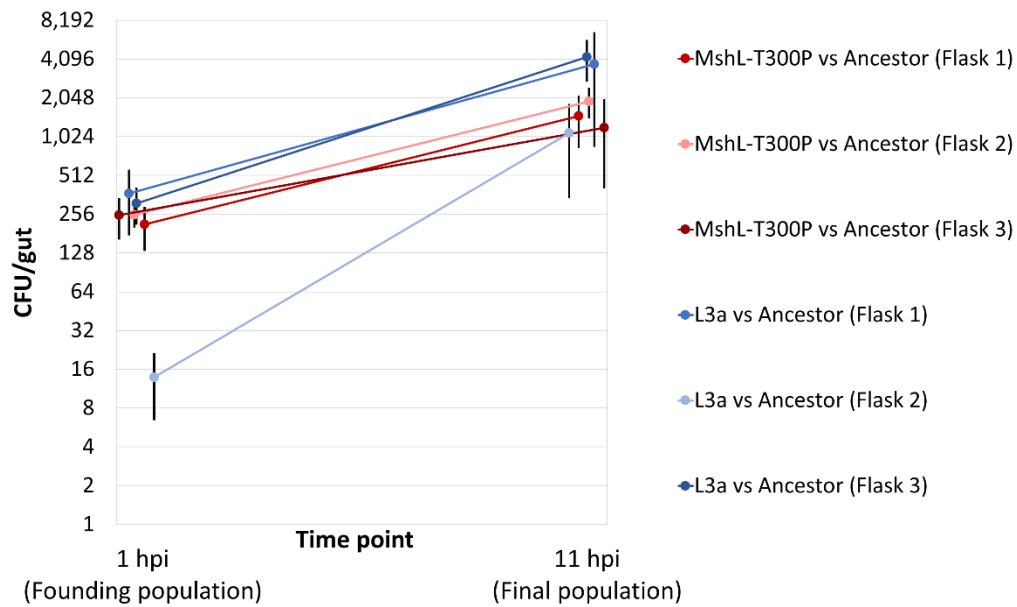1   *Supplements:*

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4   **Figure S1: Location and representation of MshL amino acid 300 in orthologous proteins.**   A) Shown is
5   the substructure of ExeD from residues 209-479. The enlarged residue, depicted in a beta-sheet,
6   indicates the position of an ExeD alanine at residue 243 which corresponded to the MshL threonine at
7   residue 300 when the two proteins were aligned. B) WebLogo 3.6.0 (Crooks et al., 2004) was used to
8   depict the amino acid representation among 1247 orthologs around the 300th residue position of MR-1's
9   MshL protein. An arrow points to the 300th position (site of MshL-T300P mutation). The height of the
10   stacks at each position indicates the level of conservation which can be influenced by a difference in
11   amino acids represented, or the absence of an amino acid in certain orthologs. The height of symbols
12   featured within each stack indicates the relative frequency of an amino acid at that position. Stack
13   widths indicate the proportion of orthologs without gaps for each position (stacks with values for all
14   1247 orthologs will have the widest widths). Symbol colors: green: polar, purple: neutral, blue: basic,
15   red: acidic, black: hydrophobic.

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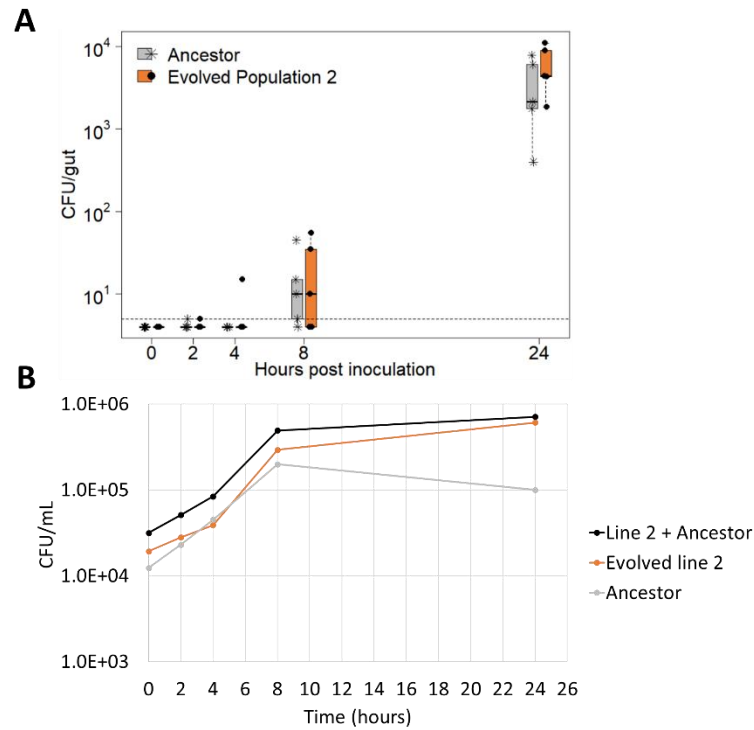| Amino Acid	Orthologous representation corresponding to MR-1 MshL-300
Arginine	1.7%
Histidine	0.1%
Lysine	0.3%
Aspartic acid	0.2%
Glutamic acid	4.8%
Serine	3.7%
Threonine	79.3%
Asparagine	3.4%
Glutamine	3.2%
Cysteine	0.0%
Glycine	0.5%
Proline	0.0%
Alanine	1.7%
Isoleucine	0.5%
Leucine	0.0%
Methionine	0.0%
Phenylalanine	0.0%
Tryptophan	0.0%
Tyrosine	0.0%
Valine	0.6%

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20 **Table S1: MR-1 MshL-300 representation.** The amino acid representation of 1247 MshL orthologues at
21 MR-1 MshL-300 after alignment to MR-1 MshL. Positions 285-315 of MR-1 MshL were used to generate
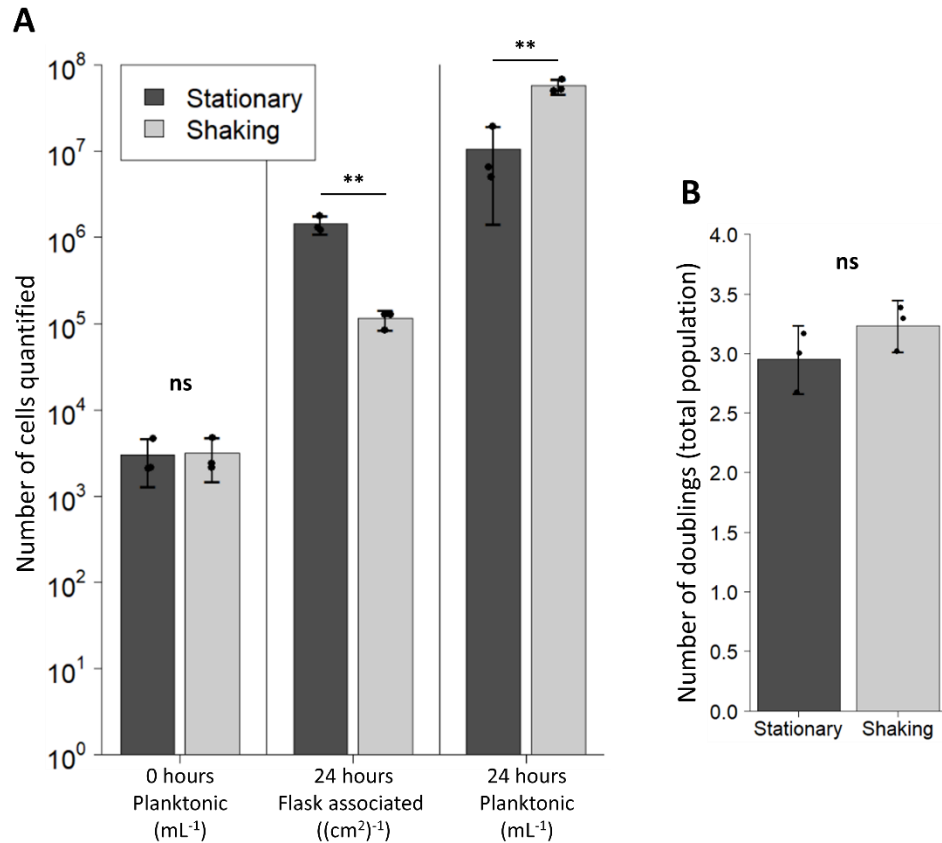22 a list of orthologs that shared 70-90 percent identity.

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Figure S2: ***In vivo* populations dynamics.** Shown is the mean number of colony-forming units determined for larval guts dissected at 1 hour post inoculation (hpi; founding population) and 11 hpi (final population). Each point represents the mean value of 10 dissected guts for a single flask. Y-axis is log_2-based. Error bars represent the 95 % confidence intervals.

Table S2: Evolved mutations. Mutations for each of six replicate evolved populations after serial passage are shown. A-D indicate that four isolates were collected from each replicate population and sequenced. Red and green shading of isolates indicates that each isolate contained a neutral dTomato or green fluorescent protein tag; used to facilitate detection of selective sweeps [7]. X indicates the presence of an evolved mutation. Yellow shading indicates a mutation in the *msh* operon.

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Figure S3: Colonization and external population dynamics over time. A) Colonization density over time. A larval GF larval flask was inoculated with competition mixture containing evolved population 2 (orange boxes) and MR-1wt (gray boxes). Five larval guts were then dissected and plated at each indicated time point. Each point shown represents the colonization density of a single gut. The dotted line indicates the limit of detection. B) Same experiment as in A) except that cell densities (CFU/mL) were measured in the EM outside the larvae over the same period.

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Figure S4: Planktonic and near-flask population dynamics. A) Number of cells quantified near the flask
surface (flask associated) and in the water column (planktonic) under static and shaking conditions in
larvae-conditioned medium (LCM). Flask associated cells were quantified from images of the flask
surface. Cells in focus were counted using an automated cell tracking algorithm. Planktonic cells were
quantified from colony-forming units (CFUs) resulting from water column samples that were plated. B)
Number of total population (planktonic + flask associated) doublings in LCM under shaking and static
conditions. For A) and B), populations are the sum of the L3a isolate and ancestral populations
competing against each other.

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