Supplemental information

A poliovirus mutant with reduced LPS binding reveals a key role for microbiota-mediated stabilization of an enteric virus

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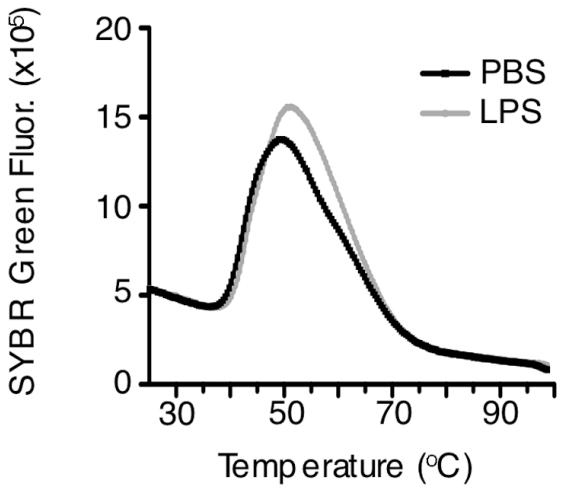
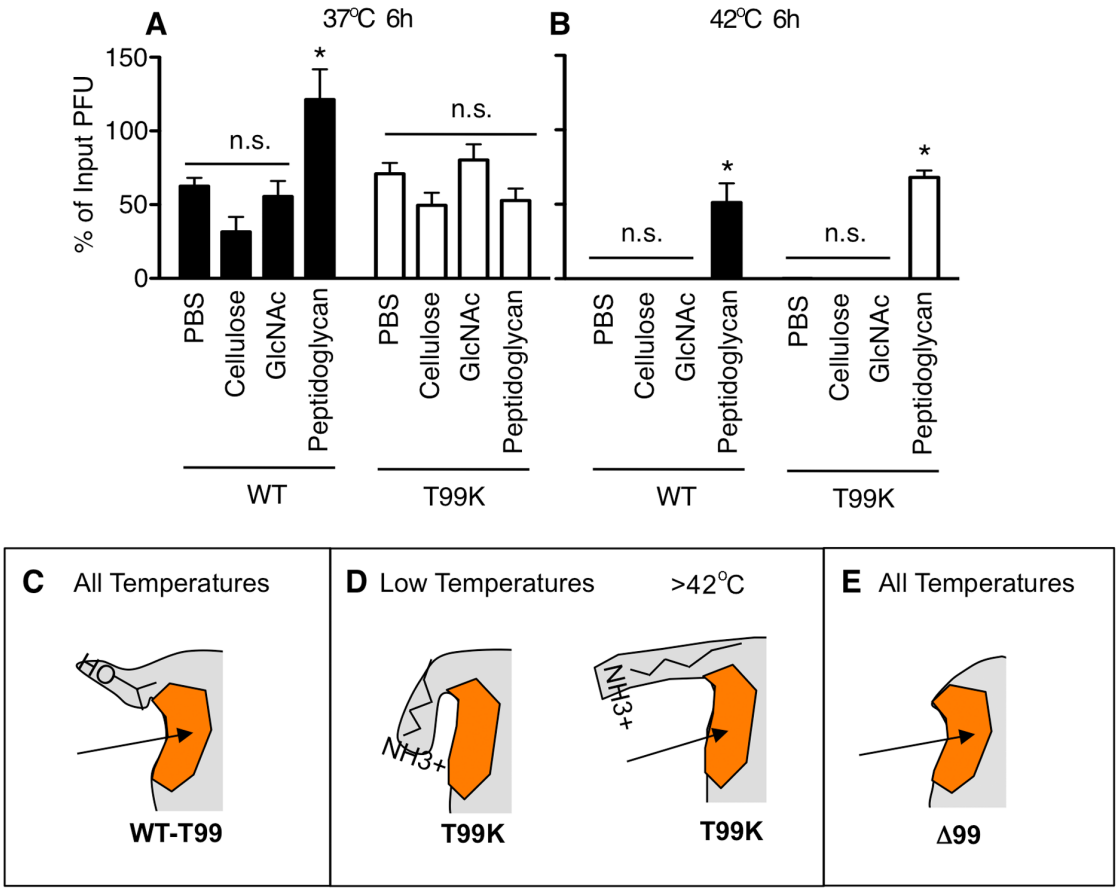


Figure S1. LPS-mediated enhancement of poliovirus thermostability, related to Figure 1. One microgram of Mahoney (WT) poliovirus was incubated with either PBS (black) or LPS (gray, 1 mg/ml) at 37˚C for 1 h. Following incubation, SYBR Green II was added and samples were placed in a real-time PCR machine. Samples were heated from 25˚C to 99˚C on a 1% stepwise gradient with fluorescence monitoring. Data are representative of 5 experiments.



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T36A	GAAGCCAGTGGACCA**G**CACACTCCAAGG
T88A	GGGGTGCATGCGTG**G**CCATTATGACCGTG
M90I	CATGCGTGACCATTAT**A**ACCGTGGATAACCCAGC
P95S	CATTATGACCGTGGATAAC**T**CAGCTTCCACCACGAATAAG
T99K	GGATAACCCAGCTTCCACCA**A**GAATAAGGATAAGCTATTTG
A106T	GGATAAGCTATTT**A**CAGTGTGGAAGATCAC
L134F	CCTATTCTAGATTTGATATGGAA**T**TTACCTTTGTGGTTACTGC
Δ99	GGATAACCCAGCTTCCACCAATAAGGATAAGCTATTTGC
T99K-NheI	GGATAACCCAGCT**AG**CACCA**AA**AATAAGGATAAGCTATTTG

Figure S2. Stability of a conditional poliovirus mutant, VP1-T99K, related to Figure 3. (A and B) Peptidoglycan-mediated enhancement of the VP1-T99K is abrogated at 37˚C, but restored when incubated at 42˚C. WT and T99K viruses were incubated at either 37˚C or 42˚C for 6 h with PBS, cellulose (1 mg/ml), GlcNAc (1 mg/ml), or peptidoglycan (1 mg/ml). Viral titers were quantified by HeLa cell plaque assay. Data are presented as mean +/- SEM; * = P<0.05; n=3-10 per condition. (C-E) Schematic representation of the putative polysaccharide-binding site (orange) on the poliovirus capsid and potential VP1-99 residue conformations at different temperatures. The arrow represents the accessibility of the polysaccharide-binding site. (F) Sense primers used to make mutations for this study. Bold nucleotides indicate changes from the WT sequence.

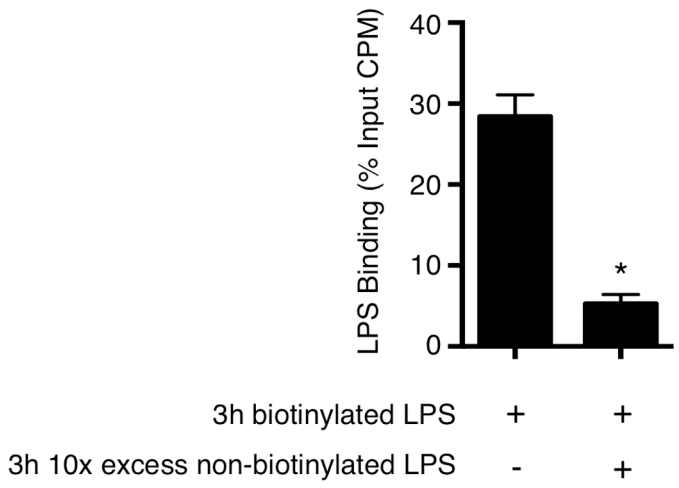

Figure S3. Poliovirus binding to LPS is reversible, related to Figure 4. ^{35}S-labeled poliovirus (10^7 PFU/7000 CPM) was incubated at 37˚C for 3 h with 150 μg/ml biotinylated LPS, followed by incubation for 3 h with streptavidin agarose resin +/- 1500 μg/ml non-biotinylated LPS, washing, and resin-associated radioactivity was quantified by scintillation counting. Data are presented as mean +/- SEM; * = P<0.05; n=3.

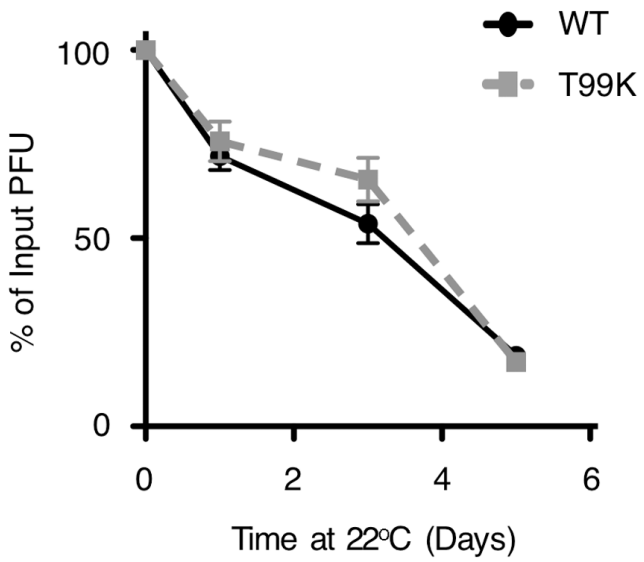

Figure S4. Thermal inactivation of WT and VP1-T99K viruses is not significantly different in the absence of bacterial polysaccharides, related to Figure 6. WT and VP1-T99K viruses were incubated with PBS at 22˚C for 5 days. Samples were collected at indicated time points and viral titers were quantified by HeLa cell plaque assay. Data are presented as mean +/- SEM; * = $P<0.05$; $n=3$ per condition.

Supplemental Experimental Procedures

Plasmid construction and mutant viruses

We created Mahoney viruses containing Sabin mutations in the capsid coding region of the Mahoney poliovirus infectious clone using Nhe I (nt 2470) and Ava I (nt 2978) restriction sites and we created Mahoney T99K virus containing an additional silent NheI restriction site at nt 2765 using AatII (nt 1122) and SnaBI (nt 2956) restriction sites. Nucleotide substitutions were created using a PCR based site-directed mutagenesis method with appropriate primers (Zheng et al., 2004). Primer sequences are listed in Fig. S2F. PCR reactions (50 ul) were carried out using *Pfu* DNA polymerase. The following conditions for the PCR were: 1) initial denaturation- 95˚C for 30 seconds, 2) denaturation- 95˚C for 30 seconds, 3) annealing 55˚C for 1 minute, 4) elongation- 68˚C for 10 minutes. Steps 2-4 were repeated 16 times. Following temperature cycling, samples were placed on ice for 2 minutes to cool the reaction. The reaction mixture was subjected to restriction digestion using DpnI to cleave the methylated template DNA. Samples were then transformed into DH5α competent cells from which plasmids were obtained. The mutant clones were confirmed by DNA sequencing. Confirmed mutants were then subcloned back into fresh type 1 Mahoney plasmid using NheI and AvaI (for Sabin mutations) or AatII and SnaBI (for T99K-Nhe) to ensure that additional unintended mutations were not present. The entire PCR-generated region of each genome was confirmed by sequencing. Plasmids were transfected into HeLa cells along with a plasmid encoding the T7 DNA-dependent RNA polymerase to produce viral stocks as previously described (Kuss et al., 2008). Molecular graphics and analysis were performed

with UCSF Chimera v. 1.6.1 (Pettersen et al., 2004) (RCSB Protein Data Bank accession number: 1HXS).

Supplemental References

Kuss, S., Etheredge, C., and Pfeiffer, J. (2008). Mulitple Host Barriers Restrict Poliovirus Trafficking in Mice. PLoS Pathog *4*, e1000082.

Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and Ferrin, T.E. (2004). UCSF Chimera--a visualization system for exploratory research and analysis. Journal of computational chemistry *25*, 1605-1612.

Zheng, L., Baumann, U., and Reymond, J.L. (2004). An efficient one-step site-directed and site-saturation mutagenesis protocol. Nucleic Acids Res *32*, e115.