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Supplemental information

Vibrio cholerae high cell density quorum sensing activates the host intestinal innate immune response

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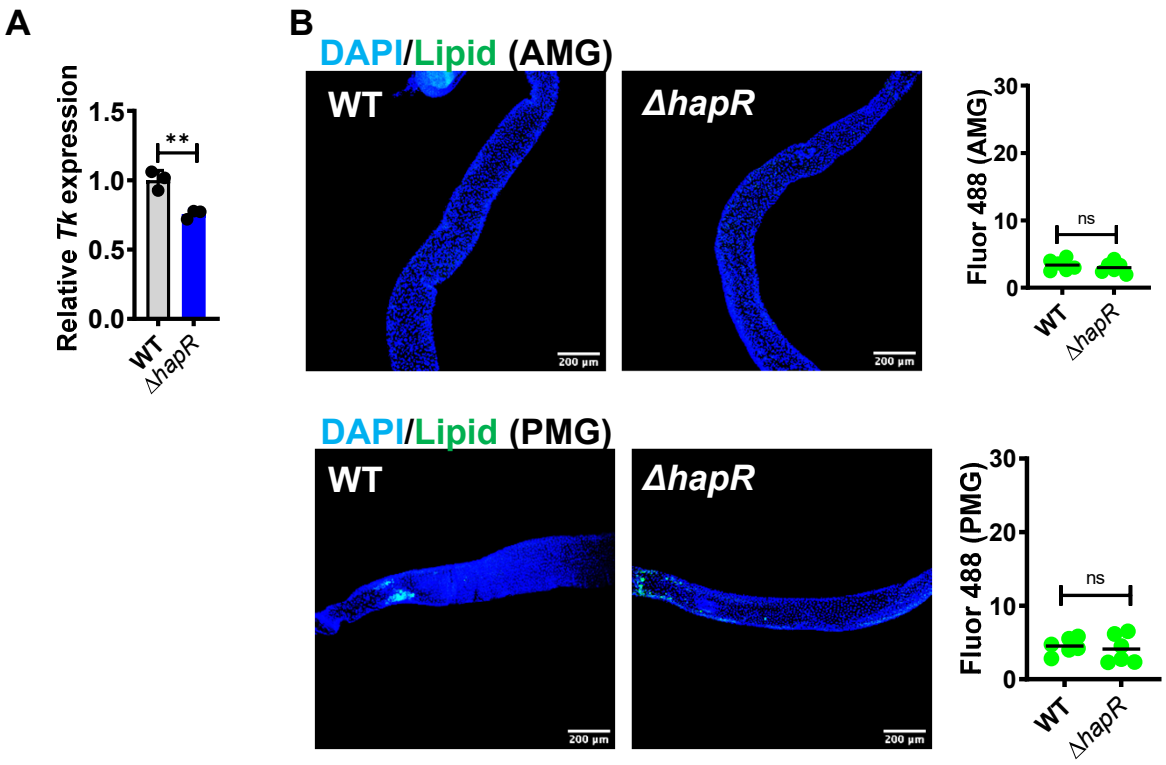


Figure S1: Decreased Tk transcription and Tk+ cells in the PMG does not result in lipid accumulation in intestinal compartments, Related to Figure 1. (A) qRT-PCR analysis of *Tk* expression in the intestines of flies infected with WT *V. cholerae* (WT) or a Δ*hapR* mutant. The mean of biological triplicates is shown. (B) Representative micrographs and fluorescence quantification (Lipid) in anterior (AMG) and posterior (PMG) midguts stained with Bodipy and DAPI. For quantification of fluorescence, the mean of at least six intestines is shown. A Student's t test was used to calculate significance. Bar=200µm. ** $p<0.01$, ns not significant.

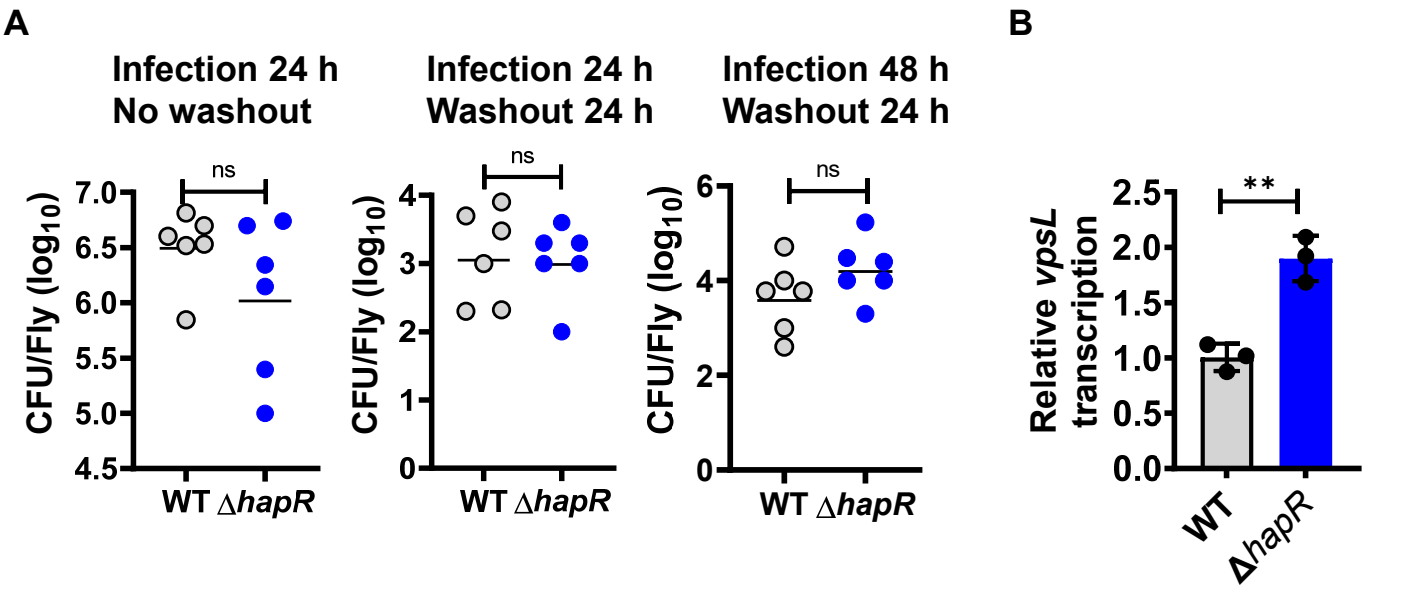


Fig S2: Stable colonization of the *Drosophila* intestine by WT and Δ*hapR* mutant *V. cholerae* is not significantly different, Related to Figure 2. (A) Female flies were colonized by ingesting LB broth inoculated with *V. cholerae* for the indicated time. A washout was performed by transferring flies to PBS. The mean pathogen burden of six individual flies is shown. (B) qRT-PCR analysis of the biofilm synthesis gene *vpsL* in flies colonized with WT *V. cholerae* or a Δ*hapR* mutant. The mean of biological triplicates is shown. Error bars represent the standard deviation. Statistical significance was assessed by a Student's t-test. ** $p<0.01$, ns not significant.

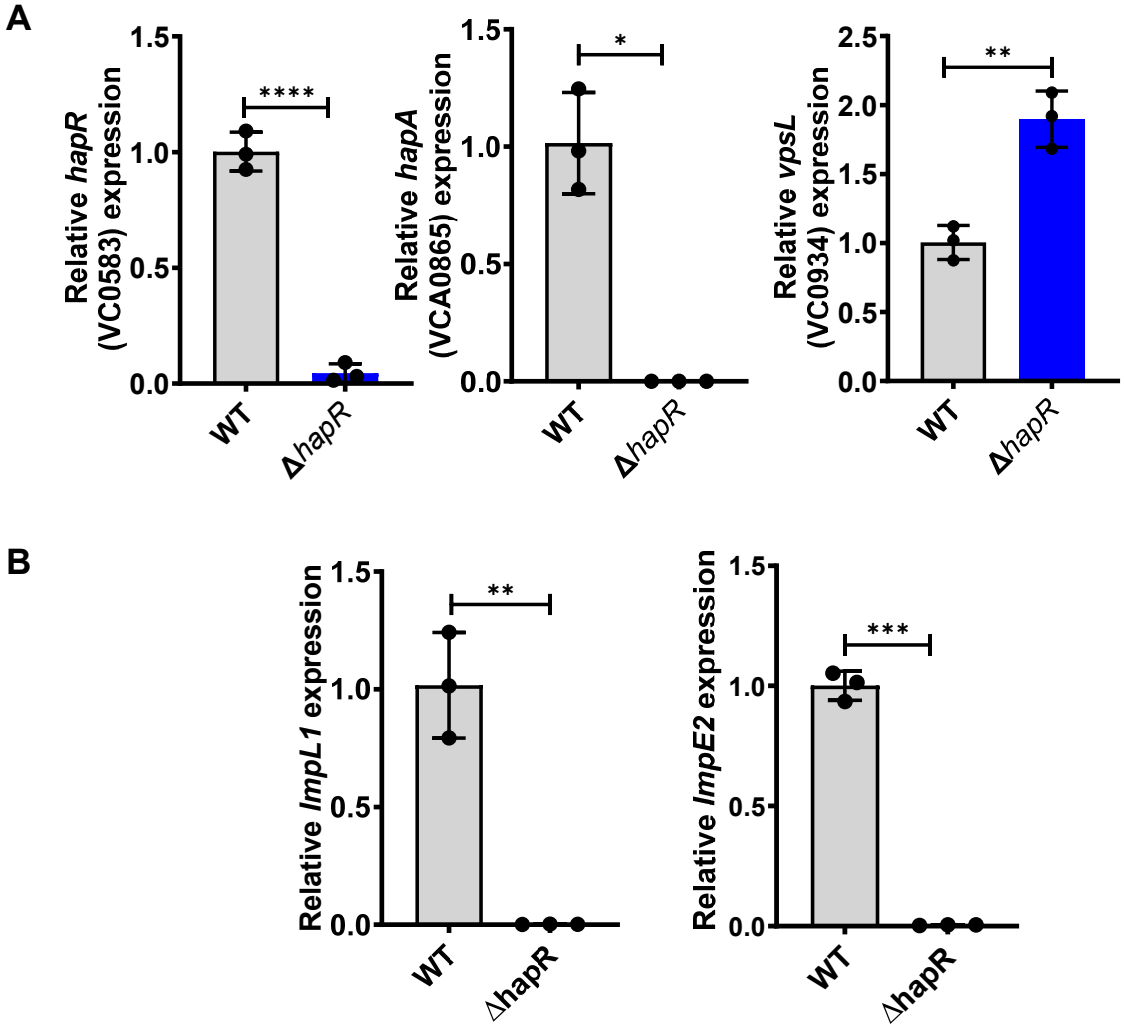


Fig S3: Validation of RNAseq results, Related to Figure 2. qRT-PCR analysis of the indicated (A) *V. cholerae* genes, and (B) 20E-regulated genes using the same mRNA used to perform RNAseq experiments. The mean of biological triplicates is shown. Error bars represent the standard deviation. Statistical significance was assessed by a Student's t-test. *** $p<0.001$, ** $p<0.01$, * $p<0.05$.

A

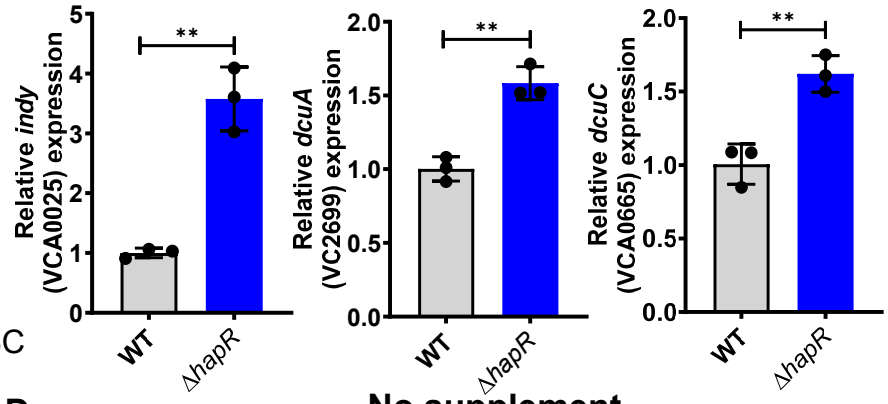
WT up

Gene locus (name)	Transporter/ Symporter
VC1659-62	amino acid ABC
VCA0757 (artM)	arginine
VCA0978	amino acid ABC
VCA1040	amino acid ABC
VC0795	Sodium/citrate
VC0910	trehalose PTS
VC1281	Cellobiose PTS
VC1325, 7-8	galactose/glucose ABC
VC1826	Mannose PTS
VC2013	Glucose PTS

B

Gene locus (name)	Transporter/ Symporter
VC0010	amino acid ABC
VC1168	proton/glutamate
VC1301	Serine/threonine
VC1422	sodium/alanine
VC2356	sodium/alanine
VCA0036	serine/threonine
VCA0088	proton/glutamate
VCA0160	tryptophan
VC1864	amino acid ABC
VC1091-3, 5	oligopeptide ABC
VC1235	sodium/carboxylate
VCA0025 (Indy)	succinate
VCA0245, 6 (PTS)	ascorbate
VCA0665 (DcuC)	succinate
VC1549-50	glycerol-3-P ABC
VCA0862	long-chain_FA

C



D

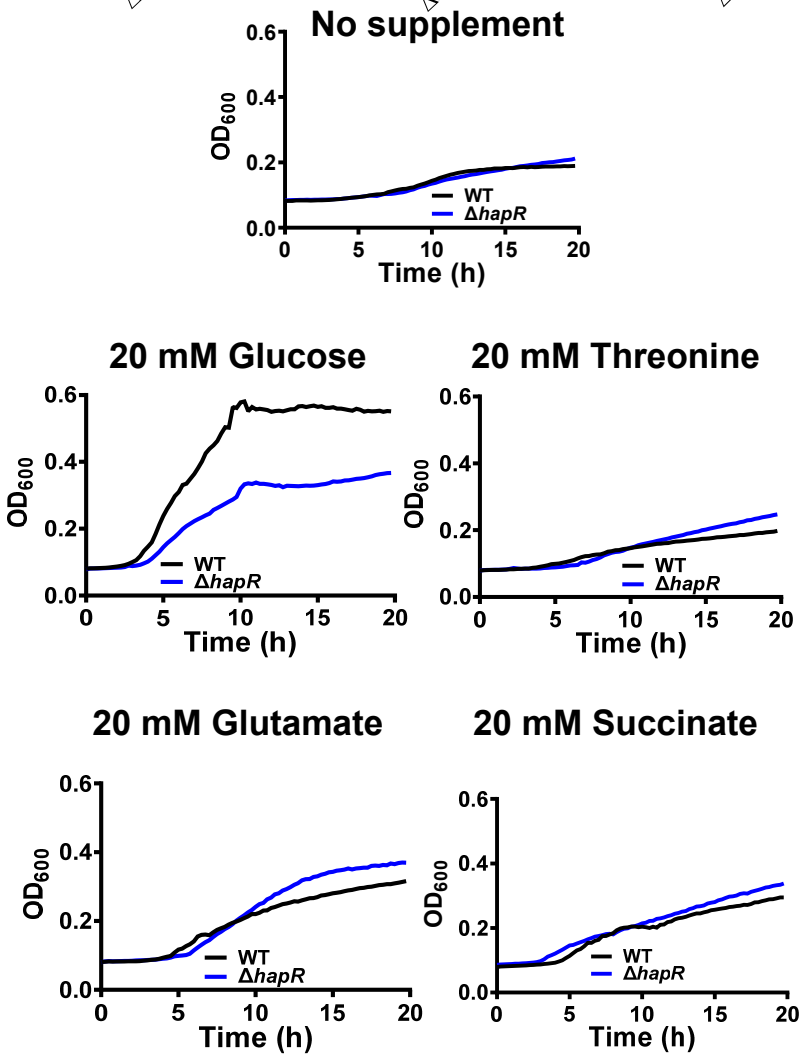


Fig S4: WT *V. cholerae* prioritizes uptake of PTS sugars at HCD while a Δ*hapR* mutant prioritizes uptake of amino acids and dicarboxylic acids, Related to Figure 3. Transporters or symporters whose transcription is increased in RNAseq analysis of (A) WT *V. cholerae* or (B) the Δ*hapR* mutant. (C) qRT-PCR analysis of the indicated proven or putative *V. cholerae* dicarboxylic acid transporters. The mean of biological triplicates is shown. Error bars represent the standard deviation. A Student's t test was used to evaluate significance. (D) Growth of WT *V. cholerae* and the Δ*hapR* mutant in minimal medium supplemented with the indicated nutrients. ** $p<0.01$.

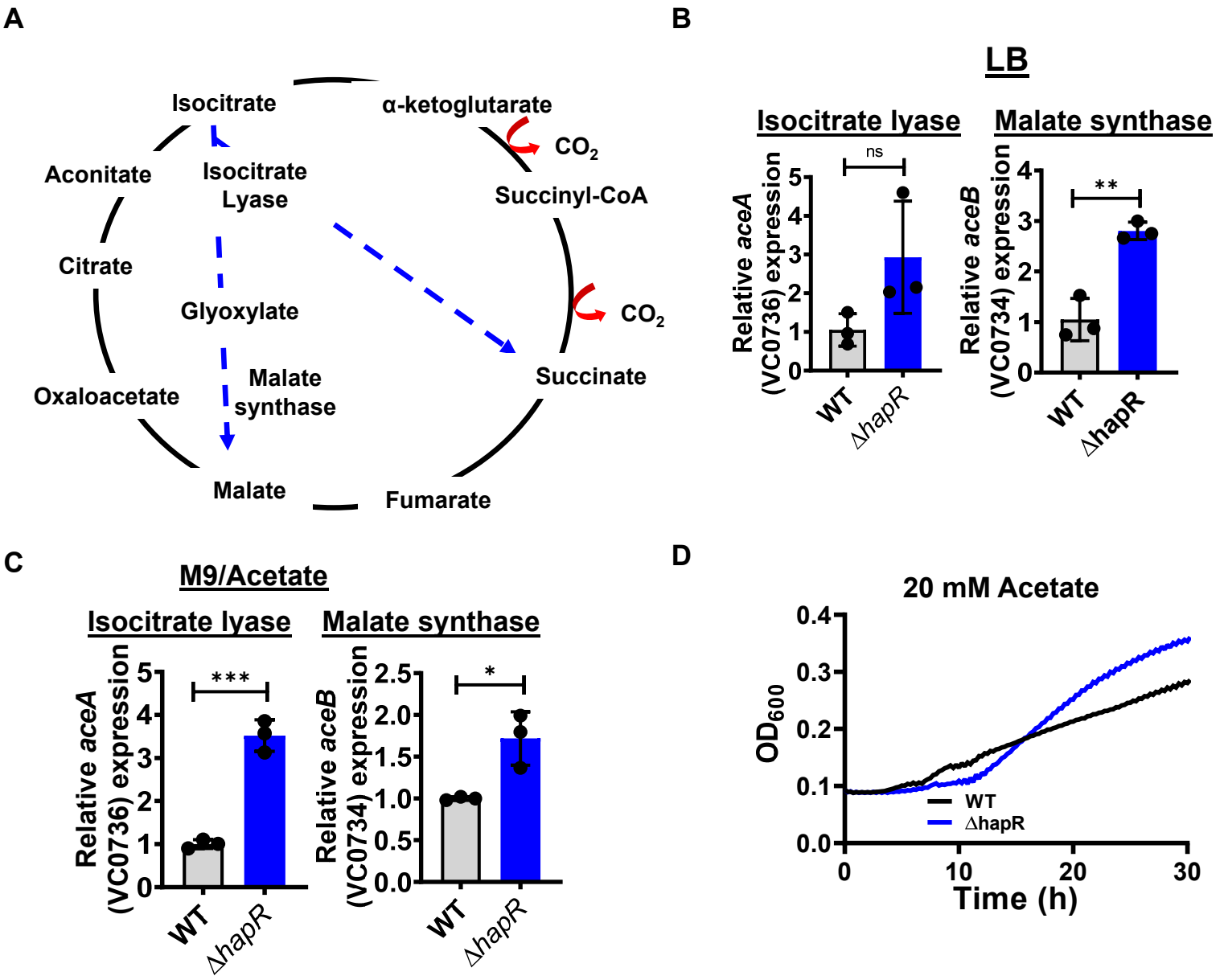


Fig S5: Mutation of *V. cholerae hapR* results in regulation indicative of increased carbon flux through the TCA cycle and glyoxylate shunt, Related to Figure 3. (A) Diagram of the TCA cycle (black lines) and glyoxylate shunt (blue dashed arrows). qRT-PCR analysis of the indicated TCA cycle and glyoxylate shunt genes in cultures of *V. cholerae* in (B) LB broth and (C) M9 minimal medium supplemented with 20 mM acetate harvested after 8 hours of growth. The mean of biological triplicates is shown. Error bars represent the standard deviation. A Student's t test was used to evaluate significance. ** $p<0.01$. (D) Growth of WT *V. cholerae* and the Δ*hapR* mutant in 20 mM acetate.

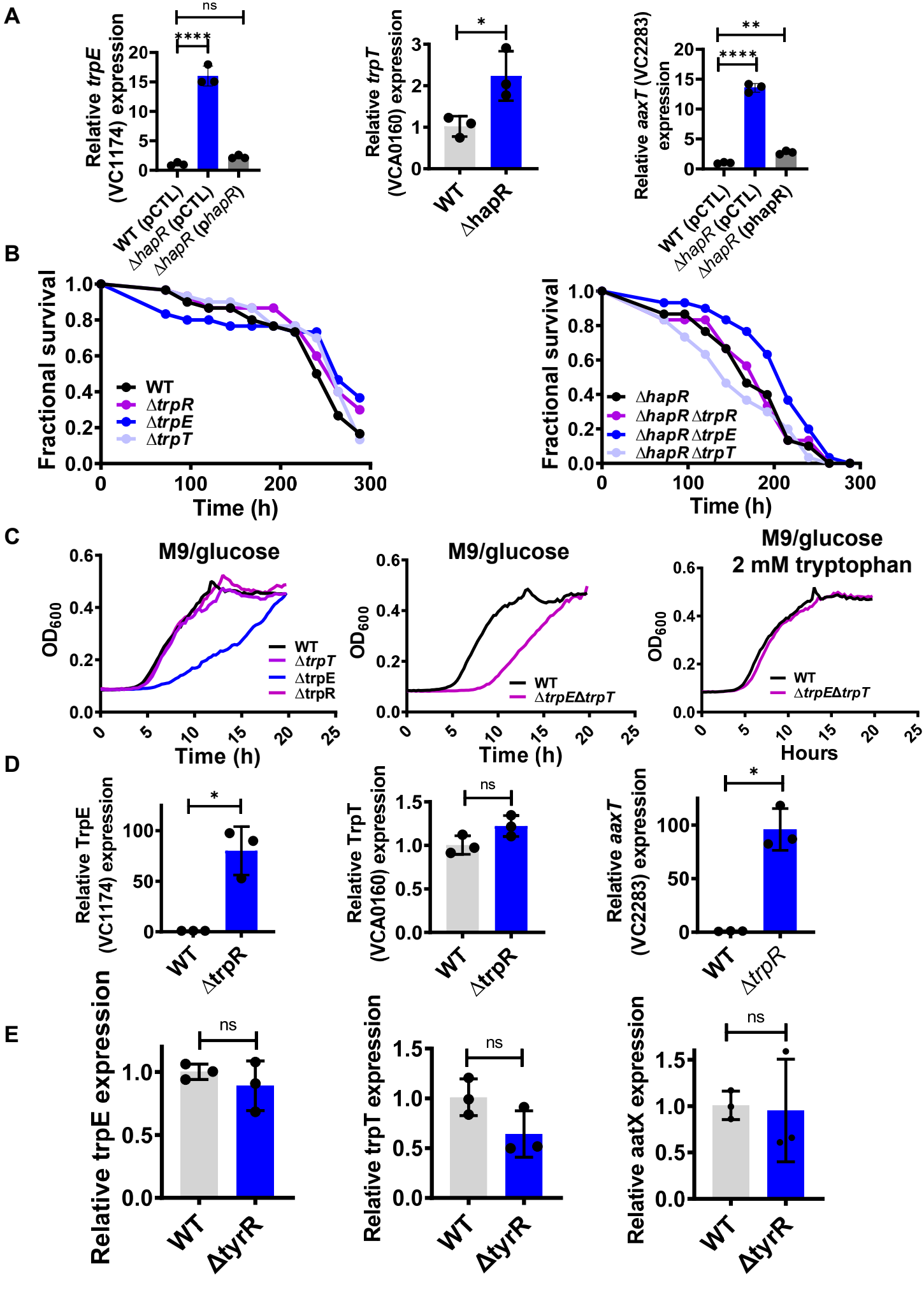

A
Relative trpE (VC1174) expression
ns
****
WT (pCTL)
ΔhapR (pCTL)
ΔhapR (phapR)
Relative trpT (VCA0160) expression
*
WT
ΔhapR
Relative aaxT (VC2283) expression
**
****
WT (pCTL)
ΔhapR (pCTL)
ΔhapR (phapR)
B
Fractional survival
Time (h)
WT
ΔtrpR
ΔtrpE
ΔtrpT
ΔhapR
ΔhapR ΔtrpR
ΔhapR ΔtrpE
ΔhapR ΔtrpT
C
M9/glucose
OD600
Time (h)
WT
ΔtrpT
ΔtrpE
ΔtrpR
M9/glucose
WT
ΔtrpEΔtrpT
M9/glucose
2 mM tryptophan
Hours
D
Relative TrpE (VC1174) expression
*
WT
ΔtrpR
Relative TrpT (VCA0160) expression
ns
Relative aaxT (VC2283) expression
*
E
Relative trpE expression
ns
WT
ΔtyrR
Relative trpT expression
ns
Relative aatX expression
ns

Fig S6: *V. cholerae* tryptophan synthesis and transport is regulated at the transcriptional level by HapR and TrpR, Related to Figure 4. (A) qRT-PCR analysis of the indicated genes in WT *V. cholerae* or a Δ*hapR* mutant. Rescue of Δ*hapR* mutant with an empty pFLAG vector (pCTL) or a pFLAG vector encoding untagged *hapR* (p*hapR*) is shown for *trpE* and *aatX* transcription. The mean of experimental triplicates is shown. Error bars represent the standard deviation. A Student's t-test with Welch's correction for unequal variances where necessary or a one-way ANOVA was used to calculate significance. (B) Survival of flies infected with WT *V. cholerae* (WT) or the indicated mutants. Log rank analysis was used to calculate significance. (C) Growth of the indicated mutants in M9 minimal medium alone or supplemented with 2 mM tryptophan. (D-E) qRT-PCR analysis of the indicated genes in WT *V. cholerae* or the indicated mutant backgrounds. The mean of experimental triplicates is shown. Error bars represent the standard deviation. A Student's t-test with Welch's correction for unequal variances where necessary was used to calculate significance. **** $p<0.0001$, *** $p<0.001$, ** $p<0.01$, * $p<0.05$, ns not significant.

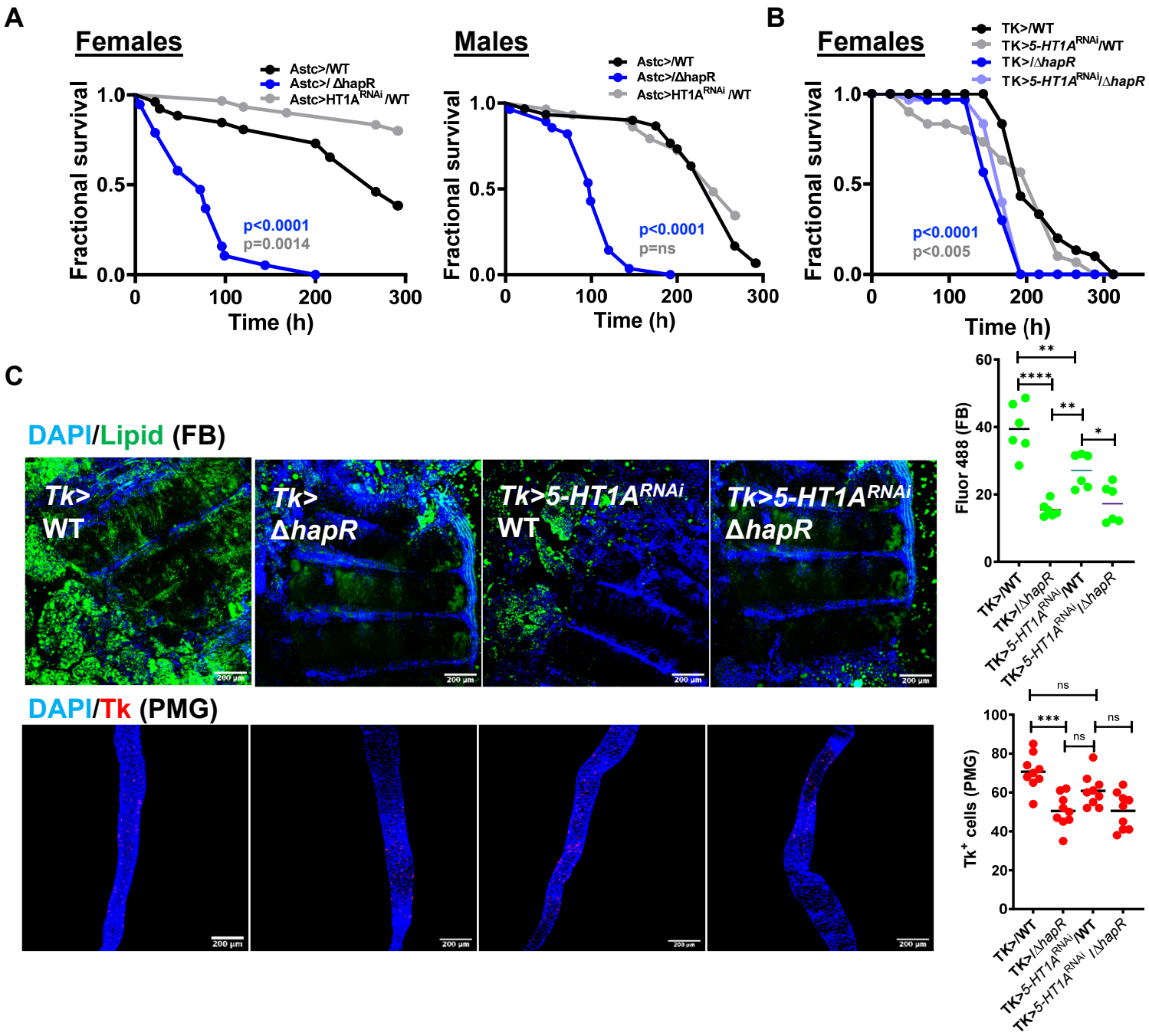


Figure S7: Activation of serotonin receptor 5-HT1A in Tk-expressing but not AstC-expressing enteroendocrine cells partially accounts for the phenotype observed in a Δ*hapR* mutant infection, Related to Figure 7. Fractional survival of EEC driver control (A) AstC>, (B) Tk> or cell type-specific *5-HT1A* knockdown male or female flies as noted orally infected with WT *V. cholerae* or a Δ*hapR* mutant. Log-rank analysis was used to calculate significance. Because these experiments were performed in tandem with those shown in Fig 7A, the Tk> controls are duplicated. (C) Representative micrographs and fluorescence quantification (Lipid) in fat bodies stained with Bodipy and DAPI and PMG immunofluorescence imaging and quantification of Tk in the intestines of *Drosophila* with genotypes as in (A) and infected with the indicated *V. cholerae* strains. Flies were dissected after 48 hours of infection. Bar=200µm. At least six intestines were evaluated. The mean is shown. A Student's t-test was used to calculate statistical significance. ****p<0.0001, ***p<0.001, **p<0.01, * p<0.05, ns not significant.