Host succinate is an activation signal for Salmonella virulence during intracellular infection

Host succinate is a signal for activation of *Salmonella* virulence during intracellular infection

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Abstract

Key to the success of intracellular pathogens is the ability to sense and respond to a changing host cell environment. Macrophages exposed to microbial products undergo metabolic changes that drive inflammatory responses. However, the role of macrophage metabolic reprogramming in bacterial adaptation to the intracellular environment has not been explored. Here, using metabolic profiling and dual RNA-seq, we show that succinate accumulation in macrophages is sensed by intracellular *Salmonella* Typhimurium (*S.*Tm) to promote antimicrobial resistance and type III secretion. *S.*Tm lacking the succinate uptake transporter DcuB display impaired survival in macrophages and in mice. Thus, *S.*Tm co-opts the metabolic reprogramming of infected macrophages as a signal that induces its own virulence and survival, providing an additional perspective on metabolic host–pathogen crosstalk.

One-sentence summary: Host metabolic reprogramming, necessary for immune activation, is co-opted by *Salmonella* to induce virulence during intracellular infection

Studies of the interactions between *Salmonella enterica* serovar Typhimurium (*S.*Tm) and macrophages have elucidated both a complex virulence program executed by the pathogen and the opposing ability of macrophages to recognize and kill invading bacteria (*1*). *S.*Tm has evolved to sense the transition from extracellular to intravacuolar environments, characterized by physiological host processes which aim to eliminate the invading pathogen (*2*). These intracellular cues provide signals that activate bacterial two-component systems, such as PhoPQ and PmrAB, which promote resistance to host antimicrobial peptides (*3*), and type III secretion systems, located on the *Salmonella* pathogenicity island 2 (SPI-2), which enables intracellular replication (*4*). Macrophage detection of invading bacteria via pattern recognition receptors results in disruption of the citric acid (TCA) cycle, which shifts host metabolism from oxidative phosphorylation toward aerobic glycolysis with consequent accumulation of several metabolic intermediates (*5*). Although it is increasingly understood that these metabolic changes are key to the regulation of host proinflammatory responses during infection (*6*), the impact of host metabolic reprogramming on intracellular pathogens is largely unknown.

To determine whether host-derived metabolites modulate the intracellular lifestyle of *S.*Tm, we sought to test their effects on *S.*Tm virulence and survival in macrophages. We utilized 2-deoxyglucose (2DG) to inhibit the shift from oxidative phosphorylation (OXPHOS) to glycolysis (*5*) in activated bone marrow–derived macrophages (BMDMs) infected with *S.*Tm (fig. S1A). We optimized an effective 2DG dose at a low concentration of 1 mM to minimize off-target effects (Fig. 1A and fig. S1, B and C). Additionally, 2DG may lead to a deficient glucose homeostasis of the bacteria that induces a stress response (*7*). 2DG treatment of *S.*Tm in culture media caused an impaired growth and induction of stress response genes, that was abolished in an *S.*Tm mutant lacking the transporter of 2DG, ManZ (*8*) (fig. S1, D to F and table S1). Thus, 2DG and the Δ*manZ* mutant can serve as a tractable model to study the impact of macrophage metabolic reprogramming on intracellular *S.*Tm.

To assess the interplay of host metabolism and pathogen dynamics in our model, we applied a dual RNA-seq method (*9*). We curated a list of 977 macrophage genes that were induced in infected cells (table S2). Host responses to infection with the WT or Δ*manZ* were similar (fig. S2A) and 2DG did not induce transcriptional changes in uninfected (naïve) cells (fig. S2B). We classified host genes that are upregulated during infection into three classes, based on their expression changes by 2DG (Fig. 1B, fig. S2C and table S2). Importantly, class 2

composed of genes downregulated by 2DG, including genes that are differentially expressed by metabolic reprogramming, verified glycolysis inhibition by 2DG (Fig. 1C and fig. S2D).

To test the impact of inhibiting host OXPHOS-to-glycolysis shift on intracellular *S.*Tm, we curated 960 bacterial genes that were upregulated during infection and differentially expressed with 2DG treatment (fig. S3A and table S3). Expression of stress response genes in WT 2DG-treated cells (fig. S3B), and of *ripA,* part of the itaconic acid degradation operon (*10*) in Δ*manZ* strain (fig. S3C), suggested that the macrophage metabolic state is sensed by intracellular *S.*Tm. Importantly, global regulon analysis (table S4) indicated decreased expression in Δ*manZ*-infected, 2DG-treated cells of regulons that mediate intracellular survival in macrophages, most prominently the SPI-2 regulon (hypergeometric test, $P<0.007$, $Q<0.12$; Fig. 1, D and E and fig. S3, D and E). Thus, there is a clear correlation between the metabolic state of infected macrophages and *S.*Tm virulence gene expression.

We next analyzed intracellular accumulation of host metabolites during infection with *S.*Tm. Several metabolites, including primary metabolites of the TCA, were significantly changed upon *S.*Tm infection and also reduced by 2DG treatment (Fig. 2A, fig. S4A, and table S5). To test whether *S.*Tm responds to the accumulation of these host metabolites, we performed a virulence assay utilizing the antimicrobial peptide polymyxin B (PMB). Survival of a Δ*phoP* strain, that is sensitive to PMB challenge (*11*), was significantly restored only in the presence of succinate and itaconate (Fig. 2B and fig. S4B). *S.*Tm grew normally in medium supplemented with succinate, but not with itaconate, at concentrations found within activated macrophages (*12*) (fig. S4, C and D). Thus, we hypothesized that succinate serves as a signal to induce *S.*Tm virulence.

Using ^{14}C-labeled succinate, we found that *S.*Tm took up and accumulated exogenous succinate (Fig. 2C). We selected a subset of SPI-2 candidate genes whose expression correlated with metabolic remodeling of infected macrophages (from Fig. 1D). We measured induced mRNA expression and fluorescence from transcriptional reporters of these genes in succinate-containing media (Fig. 2, D and E and fig. S4E). Succinate also induced protein levels of one of these SPI-2 effectors, SseL (Fig. 2F). Finally, *S.*Tm grown in succinate-containing media showed increased survival in BMDMs compared to control (Fig. 2G). Thus, succinate is sufficient to mount a bacterial pathogenic program.

Using RNA-seq, we comprehensively analyzed the direct effects of succinate on *S.*Tm (table S6). Succinate induced the expression of several regulons known to mediate *S.*Tm virulence within macrophages (Fig. 3A, fig. S5A, and table S7). In line with the observed effect of host metabolic shift on induction of SPI-2 genes during intracellular infection, growth in succinate-containing media significantly induced expression of the SPI-2 regulon (hypergeometric test, $Q<0.001$). Furthermore, succinate induced the expression of the PmrAB regulon (hypergeometric test, $Q<0.001$), important for antimicrobial peptide resistance (*3*) (Fig. 3B). We tested whether succinate-induced PMB resistance (Fig. 2B) is mediated by PmrA-regulated genes (Fig. 3C, fig. S5B). Similar to the Δ*phoP* strain, the Δ*pmrA* strain was susceptible to PMB in control media. However, succinate exposure did not induce PMB resistance in Δ*pmrA*. Resistance was preserved in a Δ*phoP* PmrA^{c} strain, but not in a Δ*pmrA* PhoP^{C} strain. Thus, succinate-mediated PMB resistance appears to be through induction of PmrA-regulated virulence genes, which deviates from the canonical activation by PhoPQ (*13*). Succinate also induced resistance to the intracellular antimicrobial peptide, defensin (*14*) (Fig. 3D and fig. S5C), suggesting a role for succinate in protection against antimicrobial peptides during intracellular infection within macrophages.

During gut colonization, *S.*Tm can utilize succinate generated by the gut microbiota to complete the TCA cycle (*15*). To test this possibility, we generated an *S.*Tm double mutant lacking *phoP* and the succinate dehydrogenase complex, subunit A (Δ*sdhA* Δ*phoP*). Similar to Δ*phoP*, Δ*sdhA* Δ*phoP* bacteria grown with succinate showed restored resistance to PMB (Fig. 3E and fig. S5, D and E). This result is consistent with succinate acting as a signal for *S.*Tm virulence independent of its role in central metabolism.

Shifts in host succinate levels might be sensed in the bacterial outer membrane or accumulate within the bacterial cytoplasm. Preferred sugars, such as glycerol, induce carbon catabolite repression, which prevents succinate uptake (*16*). Bacteria grown with succinate alone exhibited a dose-dependent induction of the SPI-2 gene *steC*, which was lost in media containing glycerol (Fig. 4A and fig. S6, A and B). The addition of permeable succinate (diethylsuccinate) resulted in elevated *steC* expression even in the presence of glycerol, or of media from activated macrophages (fig. S6C). Thus, succinate is actively transported into bacteria in order to induce *S.*Tm pathogenicity.

Succinate transport into bacteria is mediated by C4-dicarboxylate transporters (*17*). Growth in succinate-containing media increased expression of three C4-dicarboxylate transporters (Fig. 4B). We examined intracellular survival of the three transporter mutants and measured a significantly lower survival of the Δ*dcuB* mutant (Fig. 4C and fig. S6D). Expression of *dcuB* was induced by growth in succinate-containing media and within BMDMs (fig. S6, E and F). Since genetic redundancy may mask individual transporter phenotypes (*18*), we assessed succinate uptake in a triple dcu mutant (Δ3) background. Succinate uptake was inhibited in the Δ3 mutant and was significantly restored by complementation of DcuB (Fig. 4D). Similar effects were measured for the expression of the SPI-2 effector *steC* (fig. S6G). In macrophages infected with a WT or Δ*dcuB* strains, secretion of SseL SPI-2 effector to the host cytosol was significantly reduced in the Δ*dcuB* mutant (Fig. 4E). Thus, active transport of succinate through DcuB is necessary for *S.*Tm virulence within macrophages.

To test the role of succinate uptake during in vivo infection, mice were intraperitoneally (i.p.) infected either with WT and Δ*dcuB* mutant or with a Δ*phoP* and a Δ*phoP* Δ*dcuB* mutants (Fig. 4F). There was no significant difference in the competitive index between the WT and Δ*dcuB*. By contrast, the Δ*phoP* Δ*dcuB* had significantly lower survival compared to the Δ*phoP* mutant at 48 and 96 hours after infection (Paired *t* test $P<0.012$, $P<0.0003$ respectively). Thus, the relationship between host accumulation of succinate and bacterial virulence, mediated by DcuB, is also relevant for in vivo infection.

This work indicates that *S.*Tm has co-opted the metabolic reprogramming of infected macrophages as a signal that induces its own virulence (fig. S7). Other studies reinforce the importance of host and microbiota metabolic products that promote colonization by *S.*Tm and other pathogens (*19–21*). Our study indicates that during intracellular infection, uptake of host succinate by the DcuB transporter converges on a unique pathway for virulence induction. One plausible mechanism could be by direct interaction of metabolites with bacterial proteins, as with host proteins (*5*). We suggest a model whereby bacterial virulence is regulated by sensing of TCA intermediates following macrophage metabolic shift, that may be a general theme required for the pathogenic success of diverse intracellular bacteria.

References and Notes

1. E. Mills, R. Avraham, Breaking the population barrier by single cell analysis: one host against one pathogen. *Curr. Opin. Microbiol.* **36**, 69–75 (2017).

2. D. Drecktrah, L. A. Knodler, R. Ireland, O. Steele-Mortimer, The Mechanism of Salmonella Entry Determines the Vacuolar Environment and Intracellular Gene Expression: The Mechanism of Salmonella Internalization Determines Virulence Gene Expression. *Traffic*. **7**, 39–51 (2006).

3. J. S. Gunn, The Salmonella PmrAB regulon: lipopolysaccharide modifications, antimicrobial peptide resistance and more. *Trends Microbiol.* **16**, 284–290 (2008).

4. E. Jennings, T. L. M. Thurston, D. W. Holden, Salmonella SPI-2 Type III Secretion System Effectors: Molecular Mechanisms And Physiological Consequences. *Cell Host Microbe*. **22**, 217–231 (2017).

5. D. G. Ryan, L. A. J. O'Neill, Krebs Cycle Reborn in Macrophage Immunometabolism. *Annu. Rev. Immunol.* **38**, 289–313 (2020).

6. L. E. Gleeson, F. J. Sheedy, E. M. Palsson-McDermott, D. Triglia, S. M. O'Leary, M. P. O'Sullivan, L. A. J. O'Neill, J. Keane, Cutting Edge: *Mycobacterium tuberculosis* Induces Aerobic Glycolysis in Human Alveolar Macrophages That Is Required for Control of Intracellular Bacillary Replication. *J. Immunol.* **196**, 2444–2449 (2016).

7. M. Bobrovskyy, C. K. Vanderpool, The small RNA SgrS: roles in metabolism and pathogenesis of enteric bacteria. *Front. Cell. Infect. Microbiol.* **4** (2014), doi:10.3389/fcimb.2014.00061.

8. B. Erni, B. Zanolari, H. P. Kocher, The mannose permease of Escherichia coli consists of three different proteins. Amino acid sequence and function in sugar transport, sugar phosphorylation, and penetration of phage lambda DNA. *J. Biol. Chem.* **262**, 5238–5247 (1987).

9. R. Avraham, N. Haseley, A. Fan, Z. Bloom-Ackermann, J. Livny, D. T. Hung, A highly multiplexed and sensitive RNA-seq protocol for simultaneous analysis of host and pathogen transcriptomes. *Nat. Protoc.* **11**, 1477 (2016).

10. S. Srikumar, C. Kröger, M. Hébrard, A. Colgan, S. V. Owen, S. K. Sivasankaran, A. D. S. Cameron, K. Hokamp, J. C. D. Hinton, RNA-seq Brings New Insights to the Intra-Macrophage Transcriptome of Salmonella Typhimurium. *PLOS Pathog.* **11**, e1005262 (2015).

11. Y. Shi, M. J. Cromie, F.-F. Hsu, J. Turk, E. A. Groisman, PhoP-regulated Salmonella resistance to the antimicrobial peptides magainin 2 and polymyxin B. *Mol. Microbiol.* **53**, 229–241 (2004).

12. L. Tretter, A. Patocs, C. Chinopoulos, Succinate, an intermediate in metabolism, signal transduction, ROS, hypoxia, and tumorigenesis. *Biochim. Biophys. Acta BBA - Bioenerg.* **1857**, 1086–1101 (2016).

13. L. F. F. Kox, M. M. S. M. Wösten, E. A. Groisman, A small protein that mediates the activation of a two-component system by another two-component system. *EMBO J.* **19**, 1861–1872 (2000).

14. P. I. Fields, E. A. Groisman, F. Heffron, A Salmonella locus that controls resistance to microbicidal proteins from phagocytic cells. *Science*. **243**, 1059–1062 (1989).

15. L. Spiga, M. G. Winter, T. F. de Carvalho, W. Zhu, E. R. Hughes, C. C. Gillis, C. L. Behrendt, J. Kim, D. Chessa, H. L. Andrews-Polymenis, D. P. Beiting, R. L. Santos, L. V. Hooper, S. E.

Winter, An Oxidative Central Metabolism Enables Salmonella to Utilize Microbiota-Derived Succinate. *Cell Host Microbe*. **22**, 291-301.e6 (2017).

16. J. L. Botsford, R. D. DeMoss, Catabolite Repression of Tryptophanase in Escherichia coli. *J. Bacteriol.* **105**, 303–312 (1971).

17. I. G. Janausch, E. Zientz, Q. H. Tran, A. Kröger, G. Unden, C4-dicarboxylate carriers and sensors in bacteria. *Biochim. Biophys. Acta BBA - Bioenerg.* **1553**, 39–56 (2002).

18. A. Kleefeld, B. Ackermann, J. Bauer, J. Krämer, G. Unden, The Fumarate/Succinate Antiporter DcuB of Escherichia coli Is a Bifunctional Protein with Sites for Regulation of DcuS-dependent Gene Expression. *J. Biol. Chem.* **284**, 265–275 (2009).

19. P. Thiennimitr, S. E. Winter, M. G. Winter, M. N. Xavier, V. Tolstikov, D. L. Huseby, T. Sterzenbach, R. M. Tsolis, J. R. Roth, A. J. Bäumler, Intestinal inflammation allows Salmonella to use ethanolamine to compete with the microbiota. *Proc. Natl. Acad. Sci.* **108**, 17480–17485 (2011).

20. A. Jacobson, L. Lam, M. Rajendram, F. Tamburini, J. Honeycutt, T. Pham, W. Van Treuren, K. Pruss, S. R. Stabler, K. Lugo, D. M. Bouley, J. G. Vilches-Moure, M. Smith, J. L. Sonnenburg, A. S. Bhatt, K. C. Huang, D. Monack, A Gut Commensal-Produced Metabolite Mediates Colonization Resistance to Salmonella Infection. *Cell Host Microbe*. **24**, 296-307.e7 (2018).

21. M. M. Curtis, Z. Hu, C. Klimko, S. Narayanan, R. Deberardinis, V. Sperandio, The Gut Commensal Bacteroides thetaiotaomicron Exacerbates Enteric Infection through Modification of the Metabolic Landscape. *Cell Host Microbe*. **16**, 759–769 (2014).

22. A. Rytkönen, J. Poh, J. Garmendia, C. Boyle, A. Thompson, M. Liu, P. Freemont, J. C. D. Hinton, D. W. Holden, SseL, a Salmonella deubiquitinase required for macrophage killing and virulence. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 3502–3507 (2007).

23. K. A. Datsenko, B. L. Wanner, One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. *Proc. Natl. Acad. Sci.* **97**, 6640–6645 (2000).

24. S. Porwollik, C. A. Santiviago, P. Cheng, F. Long, P. Desai, J. Fredlund, S. Srikumar, C. A. Silva, W. Chu, X. Chen, R. Canals, M. M. Reynolds, L. Bogomolnaya, C. Shields, P. Cui, J. Guo, Y. Zheng, T. Endicott-Yazdani, H.-J. Yang, A. Maple, Y. Ragoza, C. J. Blondel, C. Valenzuela, H. Andrews-Polymenis, M. McClelland, Defined Single-Gene and Multi-Gene Deletion Mutant Collections in Salmonella enterica sv Typhimurium. *PLOS ONE*. **9**, e99820 (2014).

25. S. I. Miller, J. J. Mekalanos, Constitutive expression of the phoP regulon attenuates Salmonella virulence and survival within macrophages. *J. Bacteriol.* **172**, 2485–2490 (1990).

26. O. Gal-Mor, D. L. Gibson, D. Baluta, B. A. Vallance, B. B. Finlay, A Novel Secretion Pathway of Salmonella enterica Acts as an Antivirulence Modulator during Salmonellosis. *PLOS Pathog.* **4**, e1000036 (2008).

27. F. van den Ent, J. Löwe, RF cloning: A restriction-free method for inserting target genes into plasmids. *J. Biochem. Biophys. Methods*. **67**, 67–74 (2006).

28. B. K. Coombes, N. F. Brown, Y. Valdez, J. H. Brumell, B. B. Finlay, Expression and Secretion of Salmonella Pathogenicity Island-2 Virulence Genes in Response to Acidification Exhibit

Differential Requirements of a Functional Type III Secretion Apparatus and SsaL. *J. Biol. Chem.* **279**, 49804–49815 (2004).

29. E. L. Mills, B. Kelly, A. Logan, A. S. H. Costa, M. Varma, C. E. Bryant, P. Tourlomousis, J. H. M. Däbritz, E. Gottlieb, I. Latorre, S. C. Corr, G. McManus, D. Ryan, H. T. Jacobs, M. Szibor, R. J. Xavier, T. Braun, C. Frezza, M. P. Murphy, L. A. O'Neill, Succinate Dehydrogenase Supports Metabolic Repurposing of Mitochondria to Drive Inflammatory Macrophages. *Cell*. **167**, 457-470.e13 (2016).

30. L. A. Falk, M. M. Hogan, S. N. Vogel, Bone marrow progenitors cultured in the presence of granulocyte-macrophage colony-stimulating factor versus macrophage colony-stimulating factor differentiate into macrophages with distinct tumoricidal capacities. *J. Leukoc. Biol.* **43**, 471–476 (1988).

31. S. P. Hmiel, M. D. Snavely, C. G. Miller, M. E. Maguire, Magnesium transport in Salmonella typhimurium: characterization of magnesium influx and cloning of a transport gene. *J. Bacteriol.* **168**, 1444–1450 (1986).

32. F. Wang, S. Zhang, I. Vuckovic, R. Jeon, A. Lerman, C. D. Folmes, P. P. Dzeja, J. Herrmann, Glycolytic Stimulation Is Not a Requirement for M2 Macrophage Differentiation. *Cell Metab.* **28**, 463-475.e4 (2018).

33. C. Kröger, A. Colgan, S. Srikumar, K. Händler, S. K. Sivasankaran, D. L. Hammarlöf, R. Canals, J. E. Grissom, T. Conway, K. Hokamp, J. C. D. Hinton, An Infection-Relevant Transcriptomic Compendium for Salmonella enterica Serovar Typhimurium. *Cell Host Microbe*. **14**, 683–695 (2013).

34. A. J. Westermann, K. U. Förstner, F. Amman, L. Barquist, Y. Chao, L. N. Schulte, L. Müller, R. Reinhardt, P. F. Stadler, J. Vogel, Dual RNA-seq unveils noncoding RNA functions in host–pathogen interactions. *Nature*. **529**, 496–501 (2016).

35. S. Malitsky, C. Ziv, S. Rosenwasser, S. Zheng, D. Schatz, Z. Porat, S. Ben-Dor, A. Aharoni, A. Vardi, Viral infection of the marine alga Emiliania huxleyi triggers lipidome remodeling and induces the production of highly saturated triacylglycerol. *New Phytol.* **210**, 88–96 (2016)

36. M. Salem, M. Bernach, K. Bajdzienko, P. Giavalisco, A Simple Fractionated Extraction Method for the Comprehensive Analysis of Metabolites, Lipids, and Proteins from a Single Sample. *J. Vis. Exp. JoVE* (2017), doi:10.3791/55802.

37. L. Zheng, S. Cardaci, L. Jerby, E. D. MacKenzie, M. Sciacovelli, T. I. Johnson, E. Gaude, A. King, J. D. G. Leach, R. Edrada-Ebel, A. Hedley, N. A. Morrice, G. Kalna, K. Blyth, E. Ruppin, C. Frezza, E. Gottlieb, Fumarate induces redox-dependent senescence by modifying glutathione metabolism. *Nat. Commun.* **6**, 6001 (2015).

38. A. P. R. Bally, P. Lu, Y. Tang, J. W. Austin, C. D. Scharer, R. Ahmed, J. M. Boss, NF-κB Regulates PD-1 Expression in Macrophages. *J. Immunol.* **194**, 4545–4554 (2015).

39. D. G. Russell, L. Huang, B. C. VanderVen, Immunometabolism at the interface between macrophages and pathogens. *Nat. Rev. Immunol.* **19**, 291 (2019).

40. B. Kelly, L. A. J. O'Neill, Metabolic reprogramming in macrophages and dendritic cells in innate immunity. *Cell Res.* **25**, 771–784 (2015).

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Author contributions

G.R. and R.A. designed the study. G.R., D.H., C.C.M., H.B.A., L.V., S.H.A., and S.B. performed the experiments. M.F. and E.O. performed ^{14}C succinate uptake experiments. M.I. and S.M. performed metabolomics experiments. D.Y. and N.N. analyzed the data. G.R. and R.A. wrote the manuscript.

Competing Interests

The authors declare no competing interests

Data and materials availability

All RNA-seq data have been deposited in NCBI's Gene Expression Omnibus (GEO) under the super-series accession number GSE139208. All data are available in the manuscript or the supplementary materials.

Supplementary Materials

Material and Methods

Figures S1-S7

Tables S1-S10

Supplementary references (*22-40*)

Figure legends

Figure 1: Interplay between macrophage metabolic reprogramming and *S.*Tm virulence.

(A) BMDMs were infected with *S.*Tm and treated with 2DG; basal respiration (OCR, gray) and stimulated glycolysis (ECAR, black) were measured. Error bars indicate means ± SEM of 3-6 replicates representative of three independent experiments. **(B)** Heatmap of host genes upregulated in *S.*Tm-infected BMDMs compared to uninfected controls. Genes were classified into three classes based on their expression changes with 2DG. Colorbars: relative gene expression (red–blue) and fold change between ±2DG (yellow–purple). **(C and D)** Host class 2 genes and bacterial SPI-2 genes presented as the calculated difference in expression between 2DG-treated and untreated samples. **(E)** MA plots of bacterial transcripts in 2DG-treated over untreated samples (*y*-axis) versus the average absolute read counts (*x*-axis).

Figure 2: *S.*Tm virulence is induced in response to succinate.

(A) BMDMs were infected with a Δ*manZ* strain, treated with 2DG, and intracellular levels of central metabolites were measured by mass spectrometry. **(B)** Δ*phoP* mutant grown without (control; gray) or with PMB (blue) supplemented with 10 mM of the indicated metabolites. Survival was measured by CFU. (**C**) *S.*Tm was cultured in the indicated media supplemented with ^{14}C labeled succinate, and succinate uptake was measured. Background indicates heat-killed bacteria. **(D-G)** *S.*Tm was grown in control media or media supplemented with 10 mM succinate. The expression of *steC, sseL*, and *gapA* (normalizing control) was measured by quantitative real-time PCR **(D)**. Fluorescence signal was measured from *S.*Tm strains expressing a transcriptional reporter fused to selected SPI-2 promoters (normalized to OD_{600}) **(E)**. Immunoblot of SseL protein (SseL-2HA) and GroEL (**F**). BMDMs were infected with *S.*Tm and intracellular survival was measured by CFU (**G**).
Error bars indicate means ± SEM, representative of two [(C)] or three [(B), (D-G)] independent experiments. Unpaired *t* test: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Figure 3: Succinate induces SPI-2 and antimicrobial resistance.

(A) RNA-seq analysis of *S.*Tm grown in control media or succinate-containing media. SPI-2 and PmrAB regulons were significantly induced in succinate. **(B)** mRNA expression of genes induced by succinate (taken from A) across the PMB resistance locus. **(C to E)** *S.*Tm strains were grown in the presence of PMB **(C and E)** or defensin **(D),** and survival measured by CFU. Error bars indicate means ± SEM, representative of three independent experiments. Unpaired *t* test: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

Figure 4: Uptake of host succinate is required for intracellular survival of *S.*Tm.

(A) *S.*Tm harboring a PsteC-*cfp* reporter was grown in media without or with glycerol, and succinate or diethylsuccinate as indicated, and fluorescence was measured (normalized to OD_{600}). **(B)** Expression of the C4-dicaboxylate transporter genes taken from RNA-seq data of Fig. 3A. **(C)** BMDMs were infected with the indicated *S.*Tm strains and intracellular survival was measured by CFU. (**D**) *S.*Tm strains were cultured in succinate-containing media, and succinate uptake was measured by ^{14}C labeled succinate. (**E**) Macrophages were infected with WT or Δ*dcuB* strains. SseL protein was quantified by protein immunoblot of bacterial pellet and host cell cytosol fractions. Boxplots: SseL protein abundance. **(F)** Groups of mice (boxes) were infected i.p. with either WT and Δ*dcuB* or Δ*phoP* and Δ*phoP* Δ*dcuB* mutants, and competitive index was measured by CFU.

Error bars indicate means ± SEM, representative of two [(B), (D), (F)] or three [(A), (C), (E)] independent experiments. Unpaired *t* test: *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.