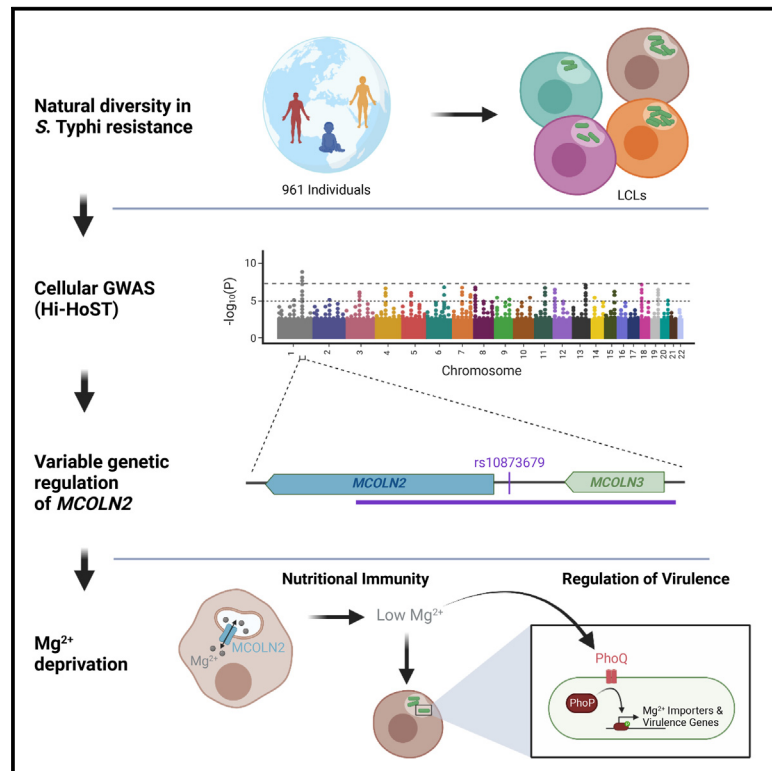


# Human variation impacting *MCOLN2* restricts *Salmonella* Typhi replication by magnesium deprivation

## Graphical abstract



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## In brief

Gibbs et al. conducted a cellular GWAS of nearly a thousand lymphoblastoid cells to identify genetic variation affecting the *MCOLN2* (TRPML2) divalent cation channel as the primary genetic determinant of variation in *Salmonella* Typhi intracellular replication. *MCOLN2* deprives *S. Typhi* of  $Mg^{2+}$ , serving as an important mechanism of nutritional immunity.

## Highlights

- Cellular GWAS revealed *MCOLN2* as an inhibitor of *S. Typhi* intracellular replication
- *MCOLN2* acts as an inward rectifying  $Mg^{2+}$  channel on endolysosomes
- *MCOLN2* deprives *S. Typhi* of  $Mg^{2+}$  to restrict growth
- *S. Typhi* replication in *MCOLN2* knockout cells depends on bacterial PhoPQ signaling



## Article

# Human variation impacting *MCOLN2* restricts *Salmonella* Typhi replication by magnesium deprivation

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## SUMMARY

Human genetic diversity can reveal critical factors in host-pathogen interactions. This is especially useful for human-restricted pathogens like *Salmonella enterica* serovar Typhi (*S. Typhi*), the cause of typhoid fever. One key defense during bacterial infection is nutritional immunity: host cells attempt to restrict bacterial replication by denying bacteria access to key nutrients or supplying toxic metabolites. Here, a cellular genome-wide association study of intracellular replication by *S. Typhi* in nearly a thousand cell lines from around the world—and extensive follow-up using intracellular *S. Typhi* transcriptomics and manipulation of magnesium availability—demonstrates that the divalent cation channel mucolipin-2 (*MCOLN2* or *TRPML2*) restricts *S. Typhi* intracellular replication through magnesium deprivation.  $Mg^{2+}$  currents, conducted through *MCOLN2* and out of endolysosomes, were measured directly using patch-clamping of the endolysosomal membrane. Our results reveal  $Mg^{2+}$  limitation as a key component of nutritional immunity against *S. Typhi* and as a source of variable host resistance.

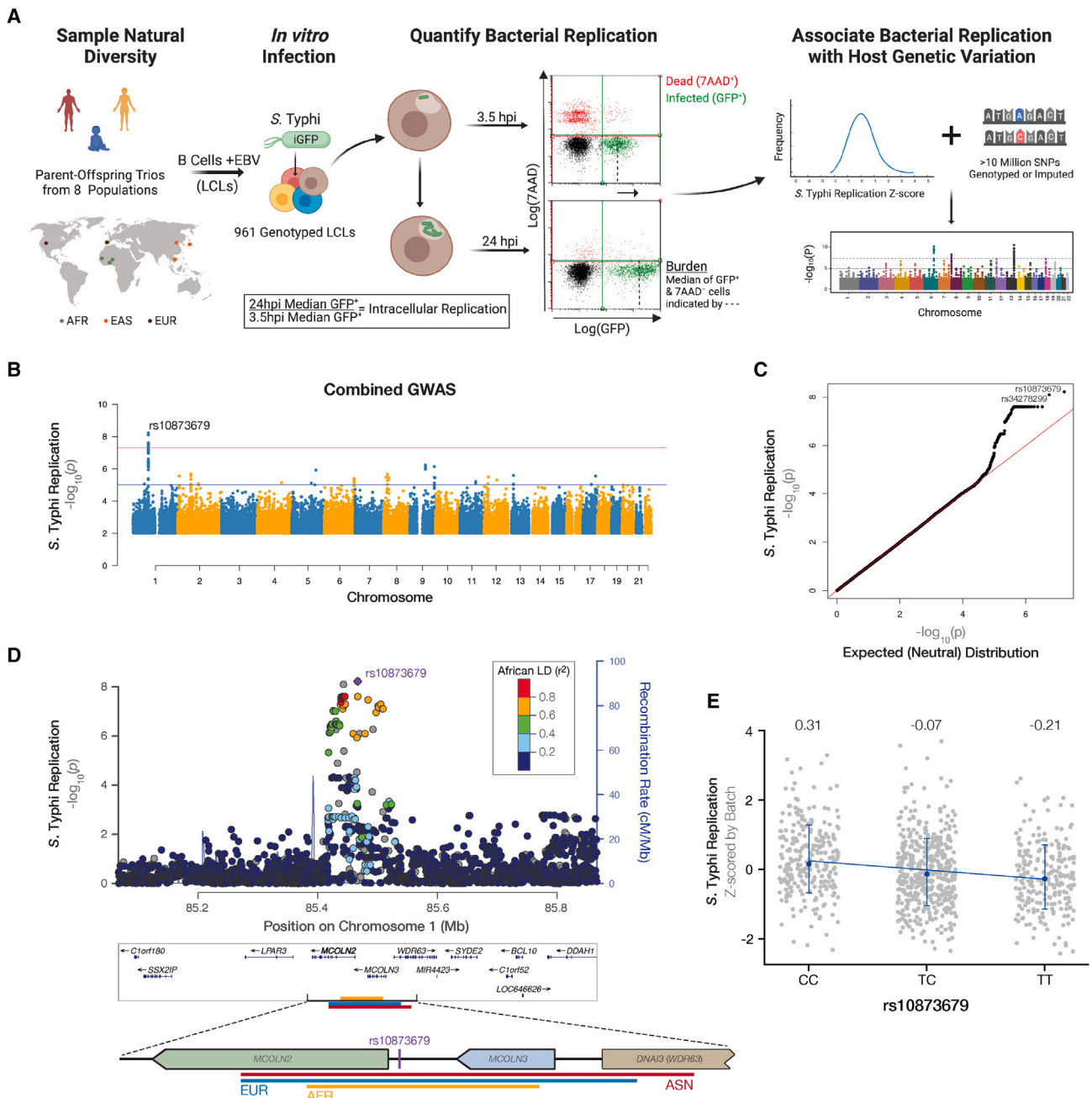
## INTRODUCTION

Genome-wide association studies (GWASs) are a powerful method to identify common genetic variants associated with risk, resistance, or other quantitative measures of infectious disease.<sup>1</sup> However, connecting variants identified by whole-organism GWAS to disease pathogenesis is often challenging—especially when it is unclear how the identified variants affect nearby genes or how these genes relate to the disease under investigation. To solve this, studies look among the disease-linked variants for those that associate with expression of nearby genes (called expression quantitative trait loci [eQTLs]), which can provide important clues, especially during stimulation with pathogens<sup>2</sup> or pathogen-associated molecular patterns.<sup>3,4</sup> A complementary approach to connect variant and disease is GWAS of cellular traits, such as our Hi-HOST (high-throughput human *in vitro* susceptibility testing)<sup>5,6</sup> platform, which associates genetic variation with quantifiable cellular traits, such as invasion,<sup>7</sup> inflammation,<sup>8</sup> and intracellular pathogen replication. As a further benefit, cellular GWASs provide control of environmental and pathogen variation, which boosts statistical power by reducing

noise. Used together, cellular GWASs of eQTLs can connect genetic variants to both altered gene expression and cellular process, explaining how the identified variation impacts clinical outcomes and facilitating subsequent mechanistic studies.

Here, we used this approach to study susceptibility to the human-restricted enteric pathogen *Salmonella enterica* ser. Typhi (*S. Typhi*), which relies on a permissive niche inside immune cells to cause the life-threatening syndrome of typhoid fever.<sup>9</sup> We discovered that the interferon-inducible<sup>10</sup> host cation channel, mucolipin-2 (*MCOLN2* or *TRPML2*), is critical for nutritional immunity against *S. Typhi*. In the dynamic competition between the host and bacteria, nutritional immunity is the ongoing effort of the host cell to restrict *Salmonella* replication by depriving it of key nutrients<sup>11–13</sup> or delivering toxic metabolites.<sup>14</sup> Nutritional immunity is well-characterized in the *Salmonella*-host competition for iron,<sup>15–18</sup> and has recently expanded to encompass competition for other key trace metal ions, such as zinc<sup>19–21</sup> and manganese.<sup>22,23</sup> Here, we demonstrate that *MCOLN2* deprives *S. Typhi* of magnesium ( $Mg^{2+}$ ), playing a major role in  $Mg^{2+}$ -based nutritional immunity for *Salmonella* replicating inside human cells.





**Figure 1. Cellular GWAS associates the rs10873679 locus with *S. Typhi* intracellular replication**

(A) Hi-HoSt cellular GWAS workflow. LCLs (Epstein-Barr virus [EBV]-immortalized B cells) were generated from eight populations sampled during the 1000 Genomes and HapMap Projects. The population locations are indicated on the map with dots coded by continental ancestry. Green dots are three populations of African (AFR) ancestry: Esan in Nigeria (ESN), Gambians in the Western Division—Mandinka (GWD), and Yoruba in Ibadan, Nigeria (YRI). Orange dots are three populations of East Asian (EAS) ancestry: Kinh in Ho Chi Minh City, Vietnam (KHV); Han Chinese in Beijing, China (CHB); and Japanese in Tokyo, Japan (JPT). Brown dots are two populations of European (EUR) ancestry: Utah residents with northern and western European ancestry from the Center d'Etude du Polymorphisme Humain (CEPH) collection (CEU) and Iberian populations in Spain (IBS). Abbreviations used: iGFP, IPTG-inducible green fluorescent protein; hpi, hours post infection; 7AAD, 7-aminoactinomycin D cell viability stain; SNP, single-nucleotide polymorphism.

(B) Manhattan plot of cellular GWAS. p values were calculated using QFAM parents on the Z scored replication ratios (orange line,  $p < 5 \times 10^{-8}$ ). The lead SNP on chromosome 1 is rs10873679 ( $p = 6 \times 10^{-9}$ ).

(C) GWAS of *S. Typhi* intracellular replication has p values lower than expected from a neutral,  $\chi^2$ , distribution (red line).

(D) A local Manhattan plot of the *S. Typhi* replication-associated locus on chromosome 1 (hg19 build) spans 400 kb up- and downstream of rs10873679. Dots for each SNP are color coded by African (AFR) linkage disequilibrium (LD) ( $r^2$ ) from 1000 genomes Nov 2014 release. A 185 kb zoom in on the *MCOLN2/3* region

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## RESULTS

We identified common human single-nucleotide polymorphisms (SNPs) associated with *S. Typhi* intracellular replication, using Hi-HOST screening and family-based GWAS analysis<sup>24</sup> of 961 lymphoblastoid cell lines (LCLs) (EBV-immortalized B cells) from eight populations (Figure 1A; Table S1). LCLs are a powerful *in vitro* model because they are karyotypically normal, and B cells are a natural site of *Salmonella* replication *in vivo*.<sup>25</sup> Because intracellular replication, or host cell permissivity, is a demonstrated proxy for *Salmonella* virulence in whole organisms,<sup>26</sup> we used variable LCL permissivity to screen for human susceptibility or resistance factors. In this screen, we defined permissivity as the ratio of bacterial burden at 24 h to 3.5 h based on median green fluorescence intensity of live intracellular *S. Typhi*. This analysis revealed a single genome-wide significant locus (lead SNP is rs10873679,  $p = 6 \times 10^{-9}$ ) on chromosome 1 (Figure 1B). A quantile-quantile plot demonstrated no overall inflation of the test statistic, with primarily rs10873679-linked SNPs deviating from the neutral distribution (Figure 1C). The association signal covers two genes in the mucolipin subfamily, *MCOLN2* and *MCOLN3* (Figure 1D). Mucolipins are a family of three inward rectifying divalent cation channels that localize to endolysosomal membranes and regulate vesicular trafficking.<sup>27</sup>

The minor (globally less common) C-allele of rs10873679 is associated with more intracellular *S. Typhi* replication (Figures 1E and S1). To link this to cellular physiology, we examined expression of *MCOLN2* and *MCOLN3* in RNA sequencing (RNA-seq) of 1000 Genomes LCLs.<sup>28</sup> The C-allele associated with less *MCOLN2* expression (Figure 2A;  $p < 2 \times 10^{-16}$ ), while rs10873679 was not associated with a significant difference in *MCOLN3* expression (Figure 2B). In confirmation, the C-allele also associated with reduced *MCOLN2* protein abundance in a quantitative mass spectrometry analysis of HapMap LCLs<sup>29</sup> (Figure 2C;  $p = 0.01$ ). In this same analysis, *MCOLN3* protein was only detected in 9 LCLs. This was insufficiently powered to draw a conclusion, although there was no evidence for association of *MCOLN3* protein with rs10873679 genotype with these limited numbers. Together, the rs10873679 C-allele's association with both more *S. Typhi* replication and less *MCOLN2* mRNA and protein suggested that *MCOLN2* restricts *S. Typhi* intracellular replication.

Strengthening this model, *MCOLN2* is upregulated in human macrophages after treatment with M1 polarizing LPS and IFN- $\gamma$ ,<sup>30</sup> which indicates that *MCOLN2* is part of the host response. Similarly, we observed *MCOLN2* induction after *S. Typhi* infection (Figure 2D). If *MCOLN2* is a restriction factor, we expected that ablating *MCOLN2* expression would increase intracellular *Salmonella* replication. Knocking down *MCOLN2*, but not *MCOLN3*, increased *S. Typhi* intracellular replication (Figure 2E), without affecting bacterial invasion or pyroptosis (Figure S2). This phenotype generalized to other human immune cells, as

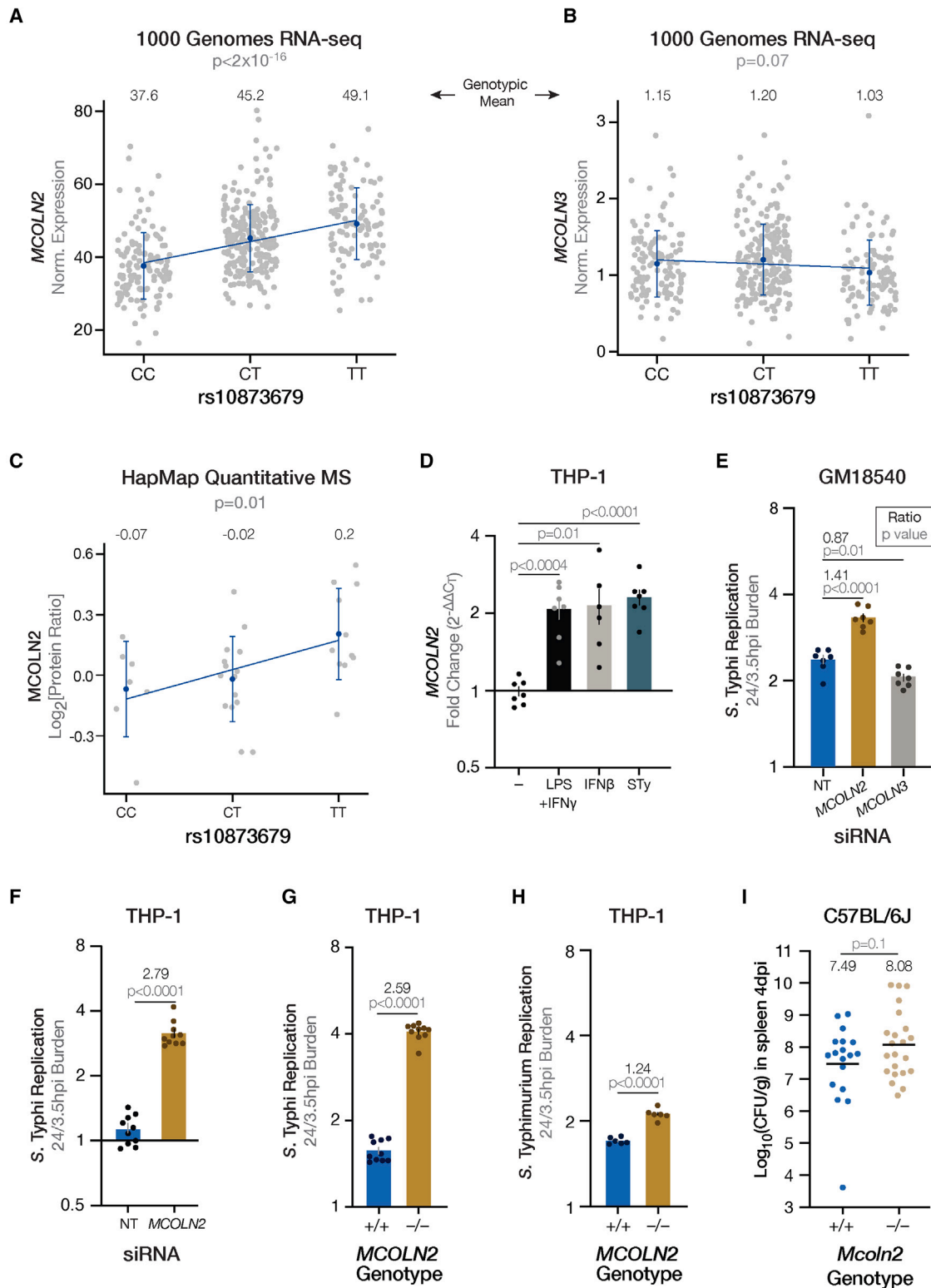
knocking down *MCOLN2* in THP-1 monocytes by RNAi (Figure 2F) or knocking out the gene using CRISPR-Cas (Figure 2G) resulted in an even greater increase in *S. Typhi* replication than in LCLs. In fact, *MCOLN2* knockout in THP-1s increased *S. Typhi* replication from 1- to 1.5-fold to 3- to 4-fold at 24 h, a large 2.5-fold increase in bacterial replication.

The rs10873679 locus was also associated with intracellular replication of *S. Typhimurium* ( $p = 8.1 \times 10^{-7}$ ; Figure S3), a serovar used to model enteric fever in mice as *S. Typhi* is human-restricted; however, the impact of reducing *MCOLN2* expression is much smaller with *S. Typhimurium* (Figure 2H). This demonstrates that, while *MCOLN2* is a key restriction factor for *S. Typhi* (knockout results in ~150% more replication), it is an accessory factor for controlling *S. Typhimurium* (knockout results in ~20% more replication). We confirmed lack of a large effect with *S. Typhimurium* by infecting susceptible C57BL/6J mice with *Mcoln2* knocked out<sup>31</sup> via intraperitoneal injection—which avoids restriction by stomach acid or variance introduced by gut microbiota—and quantified *S. Typhimurium* burden in the spleen 4 days post infection (Figure 2I). This revealed no significant difference in *S. Typhimurium* burden between *Mcoln2* genotypes, despite a modest trend of higher burden in *Mcoln2*<sup>-/-</sup> mice, which is not surprising given the small *in vitro* phenotype. This serovar difference could be explained by bacterial difference—only *S. Typhi* has the capacity to take advantage of a changed niche after *MCOLN2*'s removal—or a differential host response, in which the more immunogenic *S. Typhimurium* induces additional restriction factors that prevent it from fully exploiting *MCOLN2* knockout. Regardless, our data demonstrate that *MCOLN2* is a strong restriction factor for the human-specific serovar *S. Typhi* in cells, which underscores the value of cellular GWAS for identifying human-specific host-pathogens interactions.

To determine how *MCOLN2* reduces *S. Typhi* replication, we used the intracellular bacteria as reporters of their own environment. We conducted transcriptomics at 16 h post infection (hpi), near maximum divergence of replication inside wild-type vs. *MCOLN2*<sup>-/-</sup> THP-1s and prior to restriction in wild-type THP-1s (Figures 3A and 3B). While >2,600 bacterial genes were detected, and expression of one-quarter of the bacterial transcriptome significantly changed between late-log inoculum and 16 hpi, differences between bacteria within wild-type and *MCOLN2*<sup>-/-</sup> cells were more modest with expression of no individual bacterial gene passing significance threshold after correction for multiple testing (Table S2). Therefore, we used gene set enrichment analysis to identify *S. Typhi* processes that were upregulated in *MCOLN2*-containing wild-type THP-1s. We generated a list of 15 gene sets of physiological processes associated with virulence or divalent cation transport (Figure 3C; Table S3). Only genes regulated by the PhoP/Q two-component system<sup>32</sup> were significantly enriched (NES = -1.81 with FDR  $q = 0.004$ ) in bacteria living inside wild-type

highlights the lead SNP's location and indicates regions well linked ( $r^2 > 0.6$ ) with rs10873679 by continental ancestry: African with a 71 kb orange bar; European (EUR) with a 121 kb blue bar; and Asian (ASN) with a 138 kb red bar.

(E) The rs10873679 C-allele is associated with increased *S. Typhi* replication. Means for each genotype are indicated above the scatterplots. Bars are  $\pm$  SD. Regression slope ( $\beta = -0.26 \pm 0.04$ ) is significantly less than zero ( $p = 1.7 \times 10^{-9}$ ).



**Figure 2. Increased mucolin-2 expression restricts *S. Typhi* replication in human immune cell lines**

(A) The rs10873679 C-allele associates with less *MCOLN2* mRNA expression in 1000 Genomes Project LCLs.<sup>28</sup> Linear regression of 448 LCLs (238 females and 210 males) is significant ( $\beta = 5.8 \pm 0.6$ ;  $p < 2 \times 10^{-16}$ ; adjusted  $r^2 = 0.166$ ).

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THP-1s compared with *MCOLN2*<sup>-/-</sup> THP-1s (Figure 3D). While *S. Typhi* within *MCOLN2*<sup>-/-</sup> cells upregulate PhoP/Q targets (10.7-fold more expression than late-log), induction is greater in bacteria inside wild-type cells (13.3-fold).

To determine if PhoP/Q signaling contributes to replication in *MCOLN2* knockout cells, we infected THP-1s with the Ty800  $\Delta$ *phoPQ* strain,<sup>33</sup> which revealed that most (~75%) of the increased replication inside *MCOLN2*<sup>-/-</sup> requires intact PhoPQ signaling (Figure 3E). Chief among PhoP/Q targets is the SPI-2 T3SS, which injects effectors to maintain *Salmonella*'s intracellular niche. Removing an essential component of the SPI-2 T3SS basal body (*ssaT*) to prevent any secretion caused no change in *S. Typhi* replication within wild-type THP-1s (compare blue bars in Figure 3F). This contrasts with *S. Typhimurium*<sup>34,35</sup> but is consistent with past *S. Typhi* literature.<sup>36</sup> In contrast, replication is reduced in *MCOLN2*<sup>-/-</sup> cells, suggesting that roughly half of the PhoP/Q-dependent increase in *S. Typhi* replication depends on SPI-2 effectors (Figure 3F). This indicates the SPI-2 independence of *S. Typhi* replication in THP-1 monocytes is actually an *MCOLN2*-dependent host response that suppresses the fitness advantage provided by *S. Typhi*'s SPI-2 effectors.

Our results demonstrate that *S. Typhi* replicating inside *MCOLN2*<sup>-/-</sup> monocytes upregulate PhoP targets, which significantly boosts replication. However, in wild-type cells, the even greater induction of PhoP targets is not sufficient to increase replication, so we speculated that the PhoP upregulation was a symptom of a restrictive condition enhanced by *MCOLN2*. Three potentially restricting conditions in the SCV lead to more PhoP activity: PhoP/Q is repressed by high Mg<sup>2+</sup><sup>37</sup> and activated by cationic antimicrobial peptides<sup>38</sup> or acidification.<sup>39,40</sup> Since *MCOLN2* is a divalent cation channel, PhoP/Q was most likely responding to reduced Mg<sup>2+</sup> concentrations, which, along with Zn<sup>2+</sup>, are limited in SCVs.<sup>41,42</sup> Indeed, the PhoP-activated Mg<sup>2+</sup> importers *mgtA* and *mgtB* were both upregulated more in bacteria inside wild-type THP-1s (Figures 3D and S4). Therefore, the transcriptomics and *phoPQ* mutant infection data suggested a simple hypothesis: *MCOLN2* deprives *S. Typhi* of Mg<sup>2+</sup>.

To test this, we repleted Mg<sup>2+</sup> 2 h after infecting and measured bacterial replication (Figure 4A). Mg<sup>2+</sup> supplementation disproportionately benefited bacterial replication inside wild-type THP-1s (1.6-fold in wild-type vs. 1.2-fold in knockout THP-1s; interaction p = 0.002). Similar results were also observed with *S. Typhimurium* (Figure 4B). While our transcriptomics could also support a role for Zn<sup>2+</sup>, zinc repletion did not have interactions with the *MCOLN2* genotype, meaning it was similarly toxic to *S. Typhi* inside both *MCOLN2*<sup>-/-</sup> and wild-type THP-1 cells (Figure 4C; interaction p = 0.3). This agrees with previous findings that high concentrations of zinc are toxic to *Salmonella*.<sup>43</sup> However, *S. Typhi* inside *MCOLN2* knockout cells are not more susceptible to Zn<sup>2+</sup> repletion, so we infer that *MCOLN2* does not help *S. Typhi* resist zinc toxicity. Together, these data demonstrate that intracellular replication is held back by magnesium starvation and not zinc toxicity.

This Mg<sup>2+</sup> starvation model is supported by whole-endolysosome patch-clamp measurements. While previous studies using whole-cell patch-clamping have demonstrated that *MCOLN1* is permeable to most monovalent and divalent cations,<sup>44</sup> there has been no direct evidence showing that *MCOLN2* conducts Mg<sup>2+</sup> from the lumen of endolysosomes into the cytosol. To determine if human *MCOLN2* can conduct Mg<sup>2+</sup>, it was expressed in HEK293 cells, and endolysosomal organelles were isolated for direct patch-clamping using a previously established approach.<sup>45-47</sup> While no significant Mg<sup>2+</sup> currents were seen in non-transfected endolysosomes (Figure 4D), application of an *MCOLN2*-specific small-molecule agonist, ML2-SA1,<sup>46</sup> evoked inward Mg<sup>2+</sup> currents on intact endolysosomes isolated from *MCOLN2*-expressing cells (Figure 4E). We also observed Mg<sup>2+</sup> currents after administration of PI(3,5)P<sub>2</sub>, a putative endogenous agonist<sup>48</sup> (Figure 4F). This is especially intriguing in light of *Salmonella*'s known manipulation of phosphoinositides through the *sopB* effector<sup>49,50</sup> and our previous finding that a host protein that regulates PI(3,5)P<sub>2</sub> is associated with *Salmonella* invasion and typhoid fever risk.<sup>7</sup> These results demonstrate that *MCOLN2* conducts Mg<sup>2+</sup> and is capable of serving as a channel for Mg<sup>2+</sup> out of endolysosomes and into the cytosol.

(B) The rs10873679 genotype does not associate with *MCOLN3* mRNA expression in the same dataset ( $\beta = -0.05 \pm 0.03$ ; p = 0.07; adjusted r<sup>2</sup> = 0.005). LCLs in (A) and (B) are from four European populations (91 CEU, 92 FIN, 86 GBR, and 91 TSI) and one African population (88 YRI) and consist of 124 CC, 217 TC, and 107 TT individuals.

(C) The rs10873679 C-allele associates with less *MCOLN2* protein expression in 33 LCLs (19 female and 14 male) measured with quantitative mass spectrometry.<sup>29</sup> LCLs are from four populations: 18 CEU, 10 YRI, 4 CHB, and 1 JPT. Linear regression is significant for *MCOLN2* ( $\beta = 0.15 \pm 0.05$ , p = 0.01; adjusted r<sup>2</sup> = 0.017). In (A)–(C), bars are mean  $\pm$  SD.

(D) Both interferon treatment and *S. Typhi* (STy) infection (MOI 10 for 24 h) stimulate *MCOLN2* expression in THP-1s. Expression measured by qRT-PCR and quantified by  $\Delta\Delta C_T$  ( $\Delta C_T$  stimulated –  $\Delta C_T$  untreated). Seven replicates from three experiments. p values are from Dunnett's T3 comparison after Welch's ANOVA (p < 0.0001).

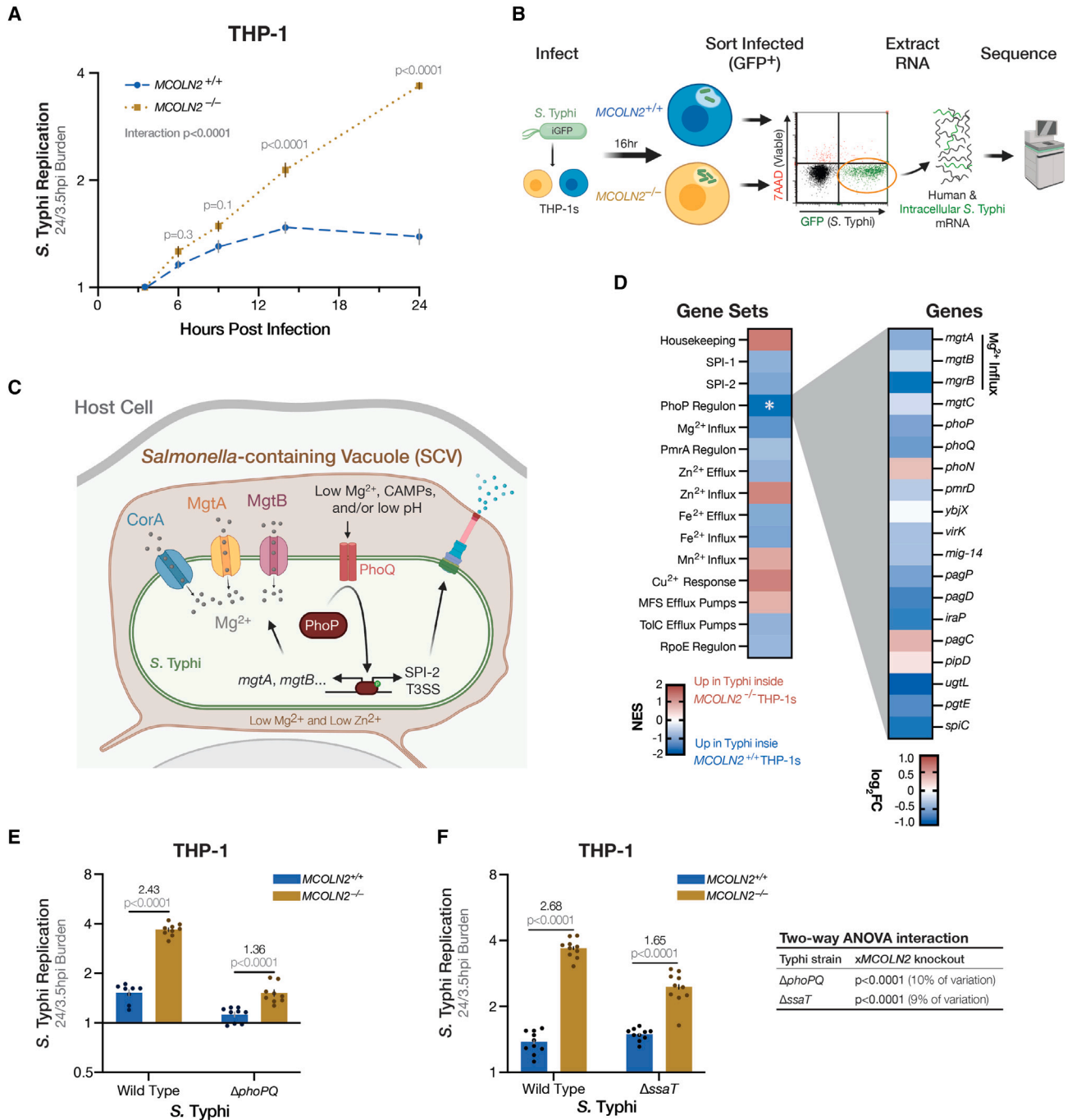
(E) In the LCL GM18540 (derived from a female CHB), *MCOLN2* knockdown increases *S. Typhi* replication, while *MCOLN3* knockdown modestly decreases *S. Typhi* replication in comparison with non-targeting (NT) siRNA. Seven replicates from three experiments. Knockdown qPCR: 0.33-fold ( $\pm 0.14$ ) of NT *MCOLN2* expression and 0.53-fold ( $\pm 0.04$ ) of NT *MCOLN3* expression. p values are from Dunnett post-hoc comparison with NT following a one-way ANOVA (main effect p < 0.0001).

(F) *MCOLN2* knockdown (0.09-fold [ $\pm 0.02$ ] of NT) increases *S. Typhi* replication in THP-1s. Ten replicates from two experiments.

(G) CRISPR-Cas9 knockout of *MCOLN2* increases *S. Typhi* replication in THP-1s. Ten replicates from two experiments.

(H) *S. Typhimurium* has a minor growth advantage in *MCOLN2* knockout THP-1s. Six replicates from two experiments. In (E)–(H), ratios are mean in siRNA-treated/NT or knockout/wild-type cells. In (D)–(H), bars are mean  $\pm$  SEM and all statistics are calculated with log<sub>2</sub>-transformed data.

(I) *Mcoln2* knockout does not significantly increase burden in C57BL/6J mice spleens 4 days post i.p. infection with 1,000 CFUs of late-log *S. Typhimurium* (14028s) tagged with p67GFP3.1. Eighteen wild types (10 female and 8 male) and 22 knockouts (13 female and 9 male) from six experiments. Lines are geometric means and log<sub>10</sub>(geo. mean) is shown above each genotype. p value calculated with log<sub>10</sub>-transformed data. Without the low outlier (log<sub>10</sub>[CFU] = 3.6; identified at Grubbs'  $\alpha = 0.01$ ), the *Mcoln2*<sup>+/+</sup> log<sub>10</sub>(geo. mean) is 7.72 and p = 0.2. In (E)–(I), p values are from Welch's t test.



**Figure 3. Intracellular *S. Typhi* replication inside *MCOLN2*<sup>-/-</sup> THP-1s depends on PhoP/Q**

(A) *MCOLN2* knockout leads to faster *S. Typhi* replication inside THP-1s. Ten replicates from three experiments, except 6 hpi is six replicates from two experiments. *MCOLN2* genotype, hpi, and their interaction are all significant sources of variation ( $p < 0.0001$ ) in a repeated measures ANOVA. Time point  $p$  values are from Šidák's post-hoc comparison of *MCOLN2*<sup>+/+</sup> and *MCOLN2*<sup>-/-</sup>.

(B) Workflow used to sequence mRNA from intracellular *S. Typhi* 16 hpi in wild-type and knockout THP-1s

(C) Diagram of *S. Typhi*'s PhoPQ-induced Mg<sup>2+</sup> importers.

(D) RNA-seq of intracellular *S. Typhi* indicates that PhoP targets are upregulated more when *MCOLN2* is present. Left: normalized enrichment score (NES) from gene set enrichment analysis of virulence- or cation-associated *S. Typhi* gene sets. Significant gene set (FDR  $q < 0.05$ ) indicated by asterisk. Right: the log<sub>2</sub>(KO/WT expression) of PhoPQ regulon genes is plotted. Sixteen of 19 genes have a negative fold change (FC), indicating higher expression in WT.

(E) *PhoPQ* is required for most of the increase in intracellular replication observed with *MCOLN2*<sup>-/-</sup> THP-1s. Nine replicates from two experiments.

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The depletion and electrophysiological evidence is further bolstered by genetic interaction of *MCOLN2* with *Salmonella*  $Mg^{2+}$  transporters. The importance of  $Mg^{2+}$  acquisition for *Salmonella* replication is underscored by its trio of  $Mg^{2+}$  uptake proteins: one constitutive, *CorA*, and two inducible, *MgtA* and *MgtB*. If knocking out *MCOLN2* increases  $Mg^{2+}$  availability, we theorized that these transporters would be necessary to uptake that extra  $Mg^{2+}$  and therefore essential for the enhanced replication inside *MCOLN2*<sup>-/-</sup> host cells. To test this, we generated a double knockout ( $\Delta mgtA\Delta mgtB$ ), which lacks the high-affinity  $Mg^{2+}$  importers used in low- $Mg^{2+}$  environments, like the  $\leq 10 \mu M$  concentration in the SCV.<sup>41</sup> In confirmation of our hypothesis, the double importer mutant is killed, instead of replicating, inside THP-1s (Figure 4G). Knocking out *MCOLN2* provides less of an advantage to the double importer mutant (increasing replication 60% in  $\Delta\Delta$  vs. 150% in wild-type *S. Typhi*; interaction  $p < 0.001$ ). This corroborates the  $Mg^{2+}$  repletion and suggests that most of the enhanced replication in *MCOLN2*<sup>-/-</sup> THP-1s depends on increasing  $Mg^{2+}$  availability.

To test if this magnesium-MCOLN2 interaction occurs *in vivo*, we infected susceptible mice with a 1:1 ratio of double knockout ( $\Delta mgtA\Delta mgtB$  or  $\Delta\Delta$ ) and wild-type *S. Typhimurium* by intraperitoneal injection. In theory, the increased  $Mg^{2+}$  availability in *Mcoln2*<sup>-/-</sup> mice would change the competitive index (CI) between double mutant and wild-type *S. Typhimurium*. As expected,  $\Delta mgtA\Delta mgtB$  *S. Typhimurium* is greatly attenuated compared with the wild type in C57BL/6J (CI = 0.024), and the double  $Mg^{2+}$ -importer mutant's attenuation is significantly more pronounced in *Mcoln2*<sup>-/-</sup> mice (CI = 0.007; Figure 4H). Based on our cellular findings, one would expect the reduced CI in *Mcoln2*<sup>-/-</sup> mice to be driven by more replication of the wild-type bacteria that can take advantage of increased  $Mg^{2+}$  availability in *Mcoln2*<sup>-/-</sup> mice; instead, we observed no significant change in wild-type bacteria replication in *Mcoln2*<sup>-/-</sup> mice ( $\Delta\Delta_{in-/-}/\Delta\Delta_{in+/+} = 0.48$  with  $p = 0.4$ ) accompanied with significantly less replication of double mutant bacteria in *Mcoln2*<sup>-/-</sup> mice ( $\Delta\Delta_{in-/-}/\Delta\Delta_{in+/+} = 0.14$  with  $p = 0.002$ ; Figure 4I). The genetic interaction of a magnesium importer mutant with murine host *Mcoln2* genotype ( $p = 0.0002$ ) leads us to conclude that murine *Mcoln2*, like human *MCOLN2*, affects  $Mg^{2+}$  accessibility by *Salmonella* during infection. However, the comparative growth disadvantage of the *S. Typhimurium* double importer mutant in *Mcoln2* knockout mice contrasting with the comparative growth advantage of wild-type *S. Typhi* in *MCOLN2*<sup>+/+</sup> human THP-1 cells (see Figure 4G) suggests that mucolipin-2's impact on  $Mg^{2+}$  availability during infection depends on context, likely including *Salmonella* serovar and host species as well as the infected cell type or tissue. Despite these differences, the *in vivo* and *in vitro* data concur that mucolipin-2 changes *Salmonella* replication by altering their access to  $Mg^{2+}$ .

In the simplest version of our model, removing human *MCOLN2* increases  $Mg^{2+}$  availability to *S. Typhi*, which relieves a nutrient limitation and directly increases bacterial replication. However,

$\sim 1/3$  of the increased bacterial replication inside *MCOLN2* knockout cells is not explained by manipulating  $Mg^{2+}$  availability or uptake. We theorized that this putatively  $Mg^{2+}$ -independent replication boost in *MCOLN2*<sup>-/-</sup> cells could still be PhoP regulated, as we had already identified other PhoP-targets, namely SPI-2 T3SS, which further benefit *S. Typhi* replication when *MCOLN2* is knocked out. To test this, we repleted  $Mg^{2+}$  after infecting THP-1s with *S. Typhi*  $\Delta phoPQ$  (Figure 4J).  $Mg^{2+}$  increased replication of  $\Delta phoPQ$  bacteria, as it partially overcomes the inability to fully upregulate *mgtA* and *mgtB*. Furthermore, the combined  $Mg^{2+}$  repletion and *phoPQ* deletion removed any discernable difference in *S. Typhi* replication between *MCOLN2* genotypes. Thus, enhanced bacterial replication in the absence of *MCOLN2* depends on both  $Mg^{2+}$ -independent effects of PhoPQ and PhoPQ-independent effects of  $Mg^{2+}$  availability (Figure 4K).

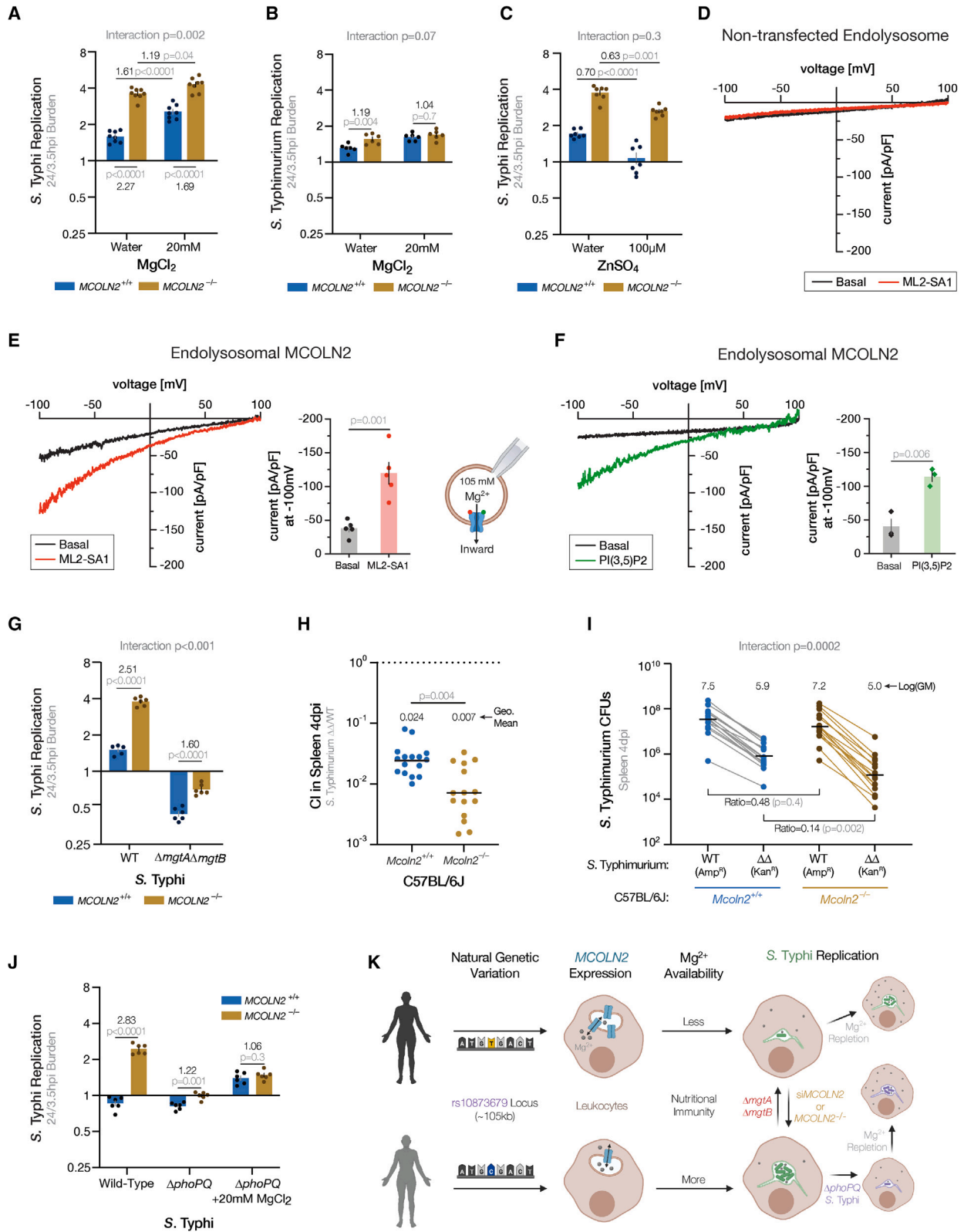
## DISCUSSION

In this report, we directly connect expression of the divalent cation channel *MCOLN2* with variable immune cell permissivity to *S. Typhi*. For *S. Typhimurium*, intracellular replication regulates outcomes in mouse models of enteric fever,<sup>26,51</sup> and, therefore, *S. Typhi* replication likely also correlates with disease outcome in humans. Unfortunately, there is no published GWAS of typhoid severity or clinical outcome and only one study on typhoid fever onset, which identified an association between the MHC region and susceptibility.<sup>52</sup> Thus, determining the clinical significance of rs10873679 in humans awaits well-powered studies for this disease phenotype. Our findings also underscore that, despite great insights gleaned from mouse models of *S. Typhimurium* infection, studies of genetic diversity using human-specific pathogens in human cells provide unique insight.

Furthermore, we showed that *MCOLN2* ablation reduced the low- $Mg^{2+}$  stress faced by intracellular *S. Typhi* based on lowered expression of  $Mg^{2+}$ -regulated PhoP targets (including key  $Mg^{2+}$  transporters) and reduced benefit of  $Mg^{2+}$  repletion. Thus, the divalent cation channel *MCOLN2* exerts restriction pressure on *S. Typhi* inside human monocytes by reducing  $Mg^{2+}$  availability, which is similar to how the divalent cation transporter *Slc11a1* (*Nramp1*) is proposed to restrict *S. Typhimurium* inside murine macrophages.<sup>53</sup> It is worth noting that C57BL/6J mice are highly susceptible to *Salmonella* due to a deleterious mutation in *Slc11a1*, which means divalent cation transport in their immune cells is already disrupted in a way that advantages *S. Typhimurium* replication.<sup>54</sup> It is possible that future work will find a greater or different effect of *Mcoln2* in mice with functional *Slc11a1*. Despite the similarity of proposed mechanisms for the effects of *MCOLN2* and *Slc11a1*, transport of  $Mg^{2+}$  by *Slc11a1* has never been demonstrated nor has human *SLC11A1* ever been shown to restrict *Salmonella* replication. This underscores the importance of our discovery that *MCOLN2* is a bona fide  $Mg^{2+}$  channel between endolysosomes and the cytosol, as it bolsters our genetic and functional evidence of  $Mg^{2+}$ -based nutritional immunity

(F) *S. Typhi*  $\Delta ssaT$  has no effect on intracellular replication in WT THP-1s and partially accounts for the requirement of *phoPQ* to achieve maximal replication in *MCOLN2*<sup>-/-</sup> THP-1s. Ten replicates from three experiments.  $p$  values in (E) and (F) are from Šidák's comparison of *MCOLN2*<sup>+/+</sup> to *MCOLN2*<sup>-/-</sup> following two-way ANOVAs finding significant main effects and interaction (all  $p < 0.0001$ ). Statistics in (A), (E), and (F) use  $\log_2$ -transformed replication ratios. Bars in (A), (E), and (F) are mean  $\pm$  SEM.





(legend on next page)

against intracellular *Salmonella*. Thus, our multi-disciplinary approach to understanding human variation, which revealed the first common human genetic difference that regulates intracellular resistance to *Salmonella*, has also led to the identification of the critical host factor that restricts *S. Typhi* by  $Mg^{2+}$  deprivation.

Identifying human *MCOLN2* as a host factor that drastically reduces *Salmonella* replication by lowering  $Mg^{2+}$  availability highlights the key role played by  $Mg^{2+}$  in nutritional immunity. This builds on a line of work identifying the sophisticated regulatory network in *S. Typhimurium* that allows it to respond to the low- $Mg^{2+}$  environment of the SCV.<sup>37,55</sup> Notably, these investigations into *Salmonella* response to low  $Mg^{2+}$  have been conducted with non-typhoidal *S. Typhimurium*. While much of this regulatory system is likely preserved in *S. Typhi*, the much greater sensitivity of *S. Typhi* to *MCOLN2* ablation suggests that some component of this low  $Mg^{2+}$  response is not conserved between the serovars. Future studies investigating this difference could reveal key serovar-specific virulence strategies.

Our finding that *MCOLN2* restricts *S. Typhi* also explains why it is an ISG, despite previous findings that it increases macrophage susceptibility to endocytosed viruses including influenza A virus (Orthomyxoviridae) and yellow fever virus (Flaviviridae).<sup>56</sup> The induction of *MCOLN2* expression in activated immune cells therefore provides two mechanisms whereby this channel could regulate infection— $Ca^{2+}$  currents regulating endocytic events and  $Mg^{2+}$  currents affecting  $Mg^{2+}$  acquisition. This identifies the *MCOLN2* locus as a possible site of balancing selection be-

tween different infectious disease pressures—viruses that use the endocytic pathway for entry might select for people with less *MCOLN2* expression, while *Salmonellae* infections might select for people with more *MCOLN2* expression. This balancing selection could explain the wide distribution of both rs10873679 alleles in populations around the world, and, ultimately, it highlights the persistent and complex power of infectious disease as an evolutionary pressure shaping human evolution.

### Limitations of the study

Our genetic association work in this study is limited to LCLs. Therefore, the association of rs10873679 with *S. Typhi* replication will need to be examined in other cell types, with varying immune cell polarization, and ultimately in human populations. Similarly, the functional studies of *MCOLN2* were consistent in LCLs and THP-1 monocytes but have not been extended to other cell types. The *MCOLN2* patch-clamp experiments were conducted using overexpression in HEK293 cells, and there may be differences with endogenous expression in immune cells. As noted above, the effects of *MCOLN2* varies across different *Salmonella enterica* serovars, and future studies will need to define the mechanistic underpinnings of these differences.

### STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

#### Figure 4. *MCOLN2* reduces *Salmonella* replication by reducing magnesium availability

- (A)  $Mg^{2+}$  supplementation partially rescues *S. Typhi* replication in *MCOLN2*<sup>-/-</sup> THP-1s. Eight replicates from two experiments.  $Mg^{2+}$  supplementation and *MCOLN2* genotype are both significant ( $p < 0.0001$ ) in two-way ANOVA. Bar plots throughout figure are mean  $\pm$  SEM.
- (B)  $Mg^{2+}$  supplementation overcomes the slight restriction of *S. Typhimurium* by *MCOLN2* in THP-1 monocytes. Six replicates from two experiments. Both supplementation ( $p = 0.0004$ ) and *MCOLN2* genotype ( $p = 0.006$ ) are significant sources of variation by two-way ANOVA.
- (C)  $Zn^{2+}$  supplementation reduces *S. Typhi* replication in THP-1 monocytes independently from *MCOLN2* genotype. Seven replicates from two experiments. In a two-way ANOVA,  $Zn^{2+}$  supplementation and *MCOLN2* genotype are significant sources of variation ( $p < 0.0001$ ), but their interaction is not ( $p = 0.3$ ). In (A)–(C), mock is the addition of an equal volume filter-sterilized DI water and p values comparing means are from Tukey's post-hoc multiple comparison tests.
- (D) No significant ML2-SA1-evoked  $Mg^{2+}$  currents were recorded from endolysosomes isolated from non-transfected HEK293 cells. Representative I-V traces of human *MCOLN2*-mediated whole-endolysosome  $Mg^{2+}$  currents before (black, basal) and after (red) treatment with the *MCOLN2* small-molecule agonist ML2-SA1.
- (E) *MCOLN2* conducts  $Mg^{2+}$  in endolysosomes when treated with ML2-SA1. A representative recording of  $Mg^{2+}$  currents from isolated endolysosomes expressing human *MCOLN2* is shown. Bar plots show mean values  $\pm$  SEM at  $-100$  mV from independent experiments. p values from paired t test. The cartoon of whole-endolysosome patch-clamp configuration indicates the direction of the inward (flow out of the vesicles)  $Mg^{2+}$  currents mediated by *MCOLN2*.
- (F) Natural *MCOLN2* agonist, PI(3,5)P2, also elicits  $Mg^{2+}$  currents through *MCOLN2*. Representative  $Mg^{2+}$  currents from endolysosomes isolated from *MCOLN2* transfected HEK293 cells, basal (black) or elicited by an application of  $10 \mu M$  PI(3,5)P2 (green). Statistical summary same as (E).
- (G) PhoPQ-induced magnesium importers MgtA and MgtB are required for half the *S. Typhi* replication benefit in *MCOLN2*<sup>-/-</sup> THP-1s. Six replicates from two experiments. In a two-way ANOVA, *MCOLN2* genotype, *mgtA/mgtB* deletion, and their interaction are all significant sources of variation ( $p < 0.0001$ ). In (B), (C), and (G), p values comparing two means are from post-hoc Šidák's multiple comparison tests.
- (H) The fitness disadvantage of an *S. Typhimurium*  $\Delta mgtA\Delta mgtB$  mutant ( $\Delta\Delta$ ) competing against wild-type (WT) *S. Typhimurium* is enhanced when competing in *Mcoln2*<sup>-/-</sup> mice. Spleens harvested 4 dpi with 1,000 CFUs of each WT and  $\Delta\Delta$  *S. Typhimurium* inoculum. Lines indicate geometric means (GM) of competitive indices (CI) and shown above each mouse genotype. One outlier (CI = 1.21) in *Mcoln2*<sup>+/+</sup> was identified by a Grubbs' test ( $\alpha = 0.0001$ ) and removed. p value from Welch's t test. Outlier test and t test calculated on  $\log_{10}$ (CFUs). Sixteen *Mcoln2*<sup>+/+</sup> mice (10 female and 6 male) and 15 *Mcoln2*<sup>-/-</sup> mice (4 female and 11 male) from 4 experiments.
- (I) The enhanced CI in *Mcoln2*<sup>-/-</sup> mice is driven by  $\Delta\Delta$  *S. Typhimurium* replicating less inside *Mcoln2*<sup>-/-</sup> than *Mcoln2*<sup>+/+</sup> mice. Data are from the same experiments and mice as in (H) and exclude the same outlier. Diagonal lines connect CFU counts from the same mouse while horizontal black lines indicate the  $\log_{10}$ -geometric mean (GM) of each group. Ratios are GM in *Mcoln2*<sup>-/-</sup> mice over GM in *Mcoln2*<sup>+/+</sup> mice. Interaction p value is from repeated measure two-way ANOVA in which CFUs from the same mouse are paired. Mouse *Mcoln2* genotype ( $p = 0.02$ ), bacteria *mgtA/B* genotype ( $p < 0.0001$ ), and their interaction ( $p = 0.0002$ ) are all significant sources of variation. p values comparing conditions are from post-hoc Šidák's multiple comparison tests. All statistics calculated using  $\log_{10}$ (CFUs).
- (J) *MCOLN2* knockout does not benefit  $\Delta phoPQ$  *S. Typhi* replication inside THP-1s when  $Mg^{2+}$  is repleted. Six replicates from two experiments. In a three-way ANOVA,  $MgCl_2$  treatment, *phoPQ* deletion, *MCOLN2* genotype, and all two-way interactions are significant ( $p < 0.0001$ ). p values comparing two means are from post-hoc Welch's t tests.
- (K) Model of natural human genetic variation altering intracellular *S. Typhi* replication.

- **KEY RESOURCES TABLE**
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#### SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.xgen.2023.100290>.

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#### AUTHOR CONTRIBUTIONS

Conceptualization, K.D.G., L.W., J.S.B., Y.C., and D.C.K.; formal analysis, K.D.G., L.W., and C.-C.C.; investigation, K.D.G., L.W., Z.Y., C.E.A., J.S.B., Y.C., M.R.G., C.-C.C., and D.C.K.; funding acquisition, K.D.G. and D.C.K.; supervision, M.B., C.-C.C., and D.C.K.; resources, K.D.G., C.E.A., J.S.B., F.B., M.B., R.P., C.-C.C., and D.C.K.; writing – original draft, K.D.G. and D.C.K.; writing – review & editing, K.D.G., J.S.B., C.-C.C., and D.C.K.

#### DECLARATION OF INTERESTS

The authors declare no competing interests.

#### INCLUSION AND DIVERSITY

We worked to ensure diversity in experimental samples through the selection of the cell lines. We support inclusive, diverse, and equitable conduct of research.

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#### REFERENCES

1. Gibbs, K.D., Schott, B.H., and Ko, D.C. (2022). The awesome power of human genetics of infectious disease. *Annu. Rev. Genet.* *56*, 41–62. <https://doi.org/10.1146/annurev-genet-080320-010449>.
2. Barreiro, L.B., Tailleux, L., Pai, A.A., Gicquel, B., Marioni, J.C., and Gilad, Y. (2012). Deciphering the genetic architecture of variation in the immune response to *Mycobacterium tuberculosis* infection. *Proc. Natl. Acad. Sci. USA* *109*, 1204–1209. <https://doi.org/10.1073/pnas.1115761109>.
3. Fairfax, B.P., Humburg, P., Makino, S., Naranbhai, V., Wong, D., Lau, E., Jostins, L., Plant, K., Andrews, R., McGee, C., and Knight, J.C. (2014). Innate immune activity conditions the effect of regulatory variants upon monocyte gene expression. *Science* *343*, 1246949. <https://doi.org/10.1126/science.1246949>.
4. Lee, M.N., Ye, C., Villani, A.C., Raj, T., Li, W., Eisenhaure, T.M., Imboywa, S.H., Chipendo, P.I., Ran, F.A., Slowikowski, K., et al. (2014). Common genetic variants modulate pathogen-sensing responses in human dendritic cells. *Science* *343*, 1246980. <https://doi.org/10.1126/science.1246980>.
5. Ko, D.C., Shukla, K.P., Fong, C., Wasnick, M., Brittnacher, M.J., Wurfel, M.M., Holden, T.D., O’Keefe, G.E., Van Yserloo, B., Akey, J.M., and Miller, S.I. (2009). A genome-wide in vitro bacterial-infection screen reveals human variation in the host response associated with inflammatory disease. *Am. J. Hum. Genet.* *85*, 214–227. <https://doi.org/10.1016/j.ajhg.2009.07.012>.
6. Wang, L., Pittman, K.J., Barker, J.R., Salinas, R.E., Stanaway, I.B., Williams, G.D., Carroll, R.J., Balmat, T., Ingham, A., Gopalakrishnan, A.M., et al. (2018). An atlas of genetic variation linking pathogen-induced cellular traits to human disease. *Cell Host Microbe* *24*, 308–323.e6. <https://doi.org/10.1016/j.chom.2018.07.007>.
7. Alvarez, M.I., Glover, L.C., Luo, P., Wang, L., Theusch, E., Oehlers, S.H., Walton, E.M., Tram, T.T.B., Kuang, Y.L., Rotter, J.I., et al. (2017). Human genetic variation in VAC14 regulates *Salmonella* invasion and typhoid fever through modulation of cholesterol. *Proc. Natl. Acad. Sci. USA* *114*, E7746–E7755. <https://doi.org/10.1073/pnas.1706070114>.
8. Bourgeois, J.S., Wang, L., Rabino, A.F., Everitt, J., Alvarez, M.I., Awadia, S., Witichen, E.S., Garcia-Mata, R., and Ko, D.C. (2021). ARHGEF26 enhances *Salmonella* invasion and inflammation in cells and mice. *PLoS Pathog.* *17*, e1009713. <https://doi.org/10.1371/journal.ppat.1009713>.
9. Johnson, R., Mylona, E., and Frankel, G. (2018). Typhoidal *Salmonella*: distinctive virulence factors and pathogenesis. *Cell Microbiol.* *20*, e12939. <https://doi.org/10.1111/cmi.12939>.
10. Schoggins, J.W., MacDuff, D.A., Imanaka, N., Gainey, M.D., Shrestha, B., Eitson, J.L., Mar, K.B., Richardson, R.B., Ratushny, A.V., Litvak, V., et al. (2014). Pan-viral specificity of IFN-induced genes reveals new roles for cGAS in innate immunity. *Nature* *505*, 691–695.
11. Hood, M.I., and Skaar, E.P. (2012). Nutritional immunity: transition metals at the pathogen-host interface. *Nat. Rev. Microbiol.* *10*, 525–537. <https://doi.org/10.1038/nrmicro2836>.
12. Núñez, G., Sakamoto, K., and Soares, M.P. (2018). Innate nutritional immunity. *J. Immunol.* *201*, 11–18. <https://doi.org/10.4049/jimmunol.1800325>.

13. Palmer, L.D., and Skaar, E.P. (2016). Transition metals and virulence in bacteria. *Annu. Rev. Genet.* *50*, 67–91. <https://doi.org/10.1146/annurev-genet-120215-035146>.
14. Chen, M., Sun, H., Boot, M., Shao, L., Chang, S.J., Wang, W., Lam, T.T., Lara-Tejero, M., Rego, E.H., and Galán, J.E. (2020). Itaconate is an effector of a Rab GTPase cell-autonomous host defense pathway against *Salmonella*. *Science* *369*, 450–455. <https://doi.org/10.1126/science.aaz1333>.
15. Cassat, J.E., and Skaar, E.P. (2013). Iron in infection and immunity. *Cell Host Microbe* *13*, 509–519. <https://doi.org/10.1016/j.chom.2013.04.010>.
16. Nairz, M., Ferring-Appel, D., Casarrubea, D., Sonnweber, T., Viatte, L., Schroll, A., Haschka, D., Fang, F.C., Hentze, M.W., Weiss, G., and Galy, B. (2015). Iron regulatory proteins mediate host resistance to *Salmonella* infection. *Cell Host Microbe* *18*, 254–261. <https://doi.org/10.1016/j.chom.2015.06.017>.
17. Nairz, M., Fritsche, G., Brunner, P., Talasz, H., Hantke, K., and Weiss, G. (2008). Interferon-gamma limits the availability of iron for intramacrophage *Salmonella typhimurium*. *Eur. J. Immunol.* *38*, 1923–1936. <https://doi.org/10.1002/eji.200738056>.
18. Weinberg, E.D. (1975). Nutritional immunity. Host's attempt to withhold iron from microbial invaders. *JAMA* *231*, 39–41. <https://doi.org/10.1001/jama.231.1.39>.
19. Gammoh, N.Z., and Rink, L. (2017). Zinc in infection and inflammation. *Nutrients* *9*, 624. <https://doi.org/10.3390/nu9060624>.
20. Cerasi, M., Liu, J.Z., Ammendola, S., Poe, A.J., Petrarca, P., Pesciaroli, M., Pasquali, P., Raffatelli, M., and Battistoni, A. (2014). The ZupT transporter plays an important role in zinc homeostasis and contributes to *Salmonella enterica* virulence. *Metallomics* *6*, 845–853.
21. Ammendola, S., Pasquali, P., Pistoia, C., Petrucci, P., Petrarca, P., Rotilio, G., and Battistoni, A. (2007). High-affinity Zn<sup>2+</sup> uptake system ZnuABC is required for bacterial zinc homeostasis in intracellular environments and contributes to the virulence of *Salmonella enterica*. *Infect. Immun.* *75*, 5867–5876.
22. Kehl-Fie, T.E., and Skaar, E.P. (2010). Nutritional immunity beyond iron: a role for manganese and zinc. *Curr. Opin. Chem. Biol.* *14*, 218–224. <https://doi.org/10.1016/j.cbpa.2009.11.008>.
23. Papp-Wallace, K.M., and Maguire, M.E. (2006). Manganese transport and the role of manganese in virulence. *Annu. Rev. Microbiol.* *60*, 187–209.
24. Purcell, S., Sham, P., and Daly, M.J. (2005). Parental phenotypes in family-based association analysis. *Am. J. Hum. Genet.* *76*, 249–259. <https://doi.org/10.1086/427886>.
25. García-Gil, A., Lopez-Bailon, L.U., and Ortiz-Navarrete, V. (2019). Beyond the antibody: B cells as a target for bacterial infection. *J. Leukoc. Biol.* *105*, 905–913. <https://doi.org/10.1002/JLB.MR0618-225R>.
26. Fields, P.I., Swanson, R.V., Haidaris, C.G., and Heffron, F. (1986). Mutants of *Salmonella Typhimurium* that cannot survive within the macrophage are avirulent. *Proc. Natl. Acad. Sci. USA* *83*, 5189–5193.
27. Spix, B., Chao, Y.K., Abrahamian, C., Chen, C.C., and Grimm, C. (2020). TRPML cation channels in inflammation and immunity. *Front. Immunol.* *11*, 225. <https://doi.org/10.3389/fimmu.2020.00225>.
28. Lappalainen, T., Sammeth, M., Friedländer, M.R., 't Hoen, P.A.C., Monlong, J., Rivas, M.A., González-Porta, M., Kurbatova, N., Griebel, T., Ferreira, P.G., et al. (2013). Transcriptome and genome sequencing uncovers functional variation in humans. *Nature* *501*, 506–511. <https://doi.org/10.1038/nature12531>.
29. Wu, L., Candille, S.I., Choi, Y., Xie, D., Jiang, L., Li-Pook-Than, J., Tang, H., and Snyder, M. (2013). Variation and genetic control of protein abundance in humans. *Nature* *499*, 79–82. <https://doi.org/10.1038/nature12223>.
30. Martínez, F.O., Gordon, S., Locati, M., and Mantovani, A. (2006). Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J. Immunol.* *177*, 7303–7311.
31. Sun, L., Hua, Y., Vergarajauregui, S., Diab, H.I., and Puertollano, R. (2015). Novel role of TRPML2 in the regulation of the innate immune response. *J. Immunol.* *195*, 4922–4932. <https://doi.org/10.4049/jimmunol.1500163>.
32. Zwir, I., Latifi, T., Perez, J.C., Huang, H., and Groisman, E.A. (2012). The promoter architectural landscape of the *Salmonella* PhoP regulon. *Mol. Microbiol.* *84*, 463–485. <https://doi.org/10.1111/j.1365-2958.2012.08036.x>.
33. Hohmann, E.L., Oletta, C.A., Killeen, K.P., and Miller, S.I. (1996). *phoP*/*phoQ*-deleted *Salmonella typhi* (Ty800) is a safe and immunogenic single-dose typhoid fever vaccine in volunteers. *J. Infect. Dis.* *173*, 1408–1414. <https://doi.org/10.1093/infdis/173.6.1408>.
34. Schroeder, N., Henry, T., de Chastellier, C., Zhao, W., Guilhon, A.A., Govel, J.P., and Méresse, S. (2010). The virulence protein SopD2 regulates membrane dynamics of *Salmonella*-containing vacuoles. *PLoS Pathog.* *6*, e1001002. <https://doi.org/10.1371/journal.ppat.1001002>.
35. Shea, J.E., Hensel, M., Gleeson, C., and Holden, D.W. (1996). Identification of a virulence locus encoding a second type III secretion system in *Salmonella typhimurium*. *Proc. Natl. Acad. Sci. USA* *93*, 2593–2597. <https://doi.org/10.1073/pnas.93.6.2593>.
36. Forest, C.G., Ferraro, E., Sabbagh, S.C., and Daigle, F. (2010). Intracellular survival of *Salmonella enterica* serovar Typhi in human macrophages is independent of *Salmonella* pathogenicity island (SPI)-2. *Microbiology* *156*, 3689–3698.
37. García Vescovi, E., Soncini, F.C., and Groisman, E.A. (1996). Mg<sup>2+</sup> as an extracellular signal: environmental regulation of *Salmonella* virulence. *Cell* *84*, 165–174.
38. Bader, M.W., Sanowar, S., Daley, M.E., Schneider, A.R., Cho, U., Xu, W., Klevit, R.E., Le Moual, H., and Miller, S.I. (2005). Recognition of antimicrobial peptides by a bacterial sensor kinase. *Cell* *122*, 461–472.
39. Alpuche Aranda, C.M., Swanson, J.A., Loomis, W.P., and Miller, S.I. (1992). *Salmonella Typhimurium* activates virulence gene transcription within acidified macrophage phagosomes. *Proc. Natl. Acad. Sci. USA* *89*, 10079–10083. <https://doi.org/10.1073/pnas.89.21.10079>.
40. Prost, L.R., Daley, M.E., Le Sage, V., Bader, M.W., Le Moual, H., Klevit, R.E., and Miller, S.I. (2007). Activation of the bacterial sensor kinase PhoQ by acidic pH. *Mol. Cell* *26*, 165–174. <https://doi.org/10.1016/j.molcel.2007.03.008>.
41. Powers, T.R., Haeberle, A.L., Predeus, A.V., Hammarlöf, D.L., Cundiff, J.A., Saldaña-Ahuactzi, Z., Hokamp, K., Hinton, J.C.D., and Knodler, L.A. (2021). Intracellular niche-specific profiling reveals transcriptional adaptations required for the cytosolic lifestyle of *Salmonella enterica*. *PLoS Pathog.* *17*, e1009280. <https://doi.org/10.1371/journal.ppat.1009280>.
42. Röder, J., Felgner, P., and Hensel, M. (2021). Comprehensive single cell analyses of the nutritional environment of intracellular *Salmonella enterica*. *Front. Cell. Infect. Microbiol.* *11*, 624650. <https://doi.org/10.3389/fcimb.2021.624650>.
43. Kapetanovic, R., Bokil, N.J., Achard, M.E.S., Ong, C.-L.Y., Peters, K.M., Stocks, C.J., Phan, M.-D., Monteleone, M., Schroder, K., Irvine, K.M., et al. (2016). *Salmonella* employs multiple mechanisms to subvert the TLR-inducible zinc-mediated antimicrobial response of human macrophages. *FASEB J.* *30*, 1901–1912.
44. Dong, X.P., Cheng, X., Mills, E., Delling, M., Wang, F., Kurz, T., and Xu, H. (2008). The type IV mucopolipidosis-associated protein TRPML1 is an endolysosomal iron release channel. *Nature* *455*, 992–996. <https://doi.org/10.1038/nature07311>.
45. Chen, C.C., Krogsaeter, E., Butz, E.S., Li, Y., Puertollano, R., Wahl-Schott, C., Biel, M., and Grimm, C. (2020). TRPML2 is an osmo/mechanosensitive cation channel in endolysosomal organelles. *Sci. Adv.* *6*, eabb5064. <https://doi.org/10.1126/sciadv.abb5064>.
46. Plesch, E., Chen, C.C., Butz, E., Scotto Rosato, A., Krogsaeter, E.K., Yinan, H., Bartel, K., Keller, M., Robaa, D., Teupser, D., et al. (2018). Selective agonist of TRPML2 reveals direct role in chemokine release from innate immune cells. *Elife* *7*, e39720. <https://doi.org/10.7554/eLife.39720>.

47. Chen, C.C., Cang, C., Fenske, S., Butz, E., Chao, Y.K., Biel, M., Ren, D., Wahl-Schott, C., and Grimm, C. (2017). Patch-clamp technique to characterize ion channels in enlarged individual endolysosomes. *Nat. Protoc.* *12*, 1639–1658. <https://doi.org/10.1038/nprot.2017.036>.
48. Dong, X.P., Shen, D., Wang, X., Dawson, T., Li, X., Zhang, Q., Cheng, X., Zhang, Y., Weisman, L.S., Delling, M., and Xu, H. (2010). PI(3,5)P(2) controls membrane trafficking by direct activation of mucolipin Ca(2+) release channels in the endolysosome. *Nat. Commun.* *1*, 38. <https://doi.org/10.1038/ncomms1037>.
49. Hernandez, L.D., Hueffer, K., Wenk, M.R., and Galán, J.E. (2004). Salmonella modulates vesicular traffic by altering phosphoinositide metabolism. *Science* *304*, 1805–1807. <https://doi.org/10.1126/science.1098188>.
50. Walpole, G.F.W., Pacheco, J., Chauhan, N., Clark, J., Anderson, K.E., Abbas, Y.M., Brabant-Kirwan, D., Montaña-Rendón, F., Liu, Z., Zhu, H., et al. (2022). Kinase-independent synthesis of 3-phosphorylated phosphoinositides by a phosphotransferase. *Nat. Cell Biol.* *24*, 708–722. <https://doi.org/10.1038/s41556-022-00895-y>.
51. Cirillo, D.M., Valdivia, R.H., Monack, D.M., and Falkow, S. (1998). Macrophage-dependent induction of the Salmonella pathogenicity island 2 type III secretion system and its role in intracellular survival. *Mol. Microbiol.* *30*, 175–188.
52. Dunstan, S.J., Hue, N.T., Han, B., Li, Z., Tram, T.T.B., Sim, K.S., Parry, C.M., Chinh, N.T., Vinh, H., Lan, N.P.H., et al. (2014). Variation at HLA-DRB1 is associated with resistance to enteric fever. *Nat. Genet.* *46*, 1333–1336. <https://doi.org/10.1038/ng.3143>.
53. Cunrath, O., and Bumann, D. (2019). Host resistance factor SLC11A1 restricts Salmonella growth through magnesium deprivation. *Science* *366*, 995–999.
54. Malo, D., Vogan, K., Vidal, S., Hu, J., Cellier, M., Schurr, E., Fuks, A., Bumstead, N., Morgan, K., and Gros, P. (1994). Haplotype mapping and sequence analysis of the mouse Nramp gene predict susceptibility to infection with intracellular parasites. *Genomics* *23*, 51–61. <https://doi.org/10.1006/geno.1994.1458>.
55. Yeom, J., Shao, Y., and Groisman, E.A. (2020). Small proteins regulate Salmonella survival inside macrophages by controlling degradation of a magnesium transporter. *Proc. Natl. Acad. Sci. USA* *117*, 20235–20243. <https://doi.org/10.1073/pnas.2006116117>.
56. Rinckenberger, N., and Schoggins, J.W. (2018). Mucolipin-2 cation channel increases trafficking efficiency of endocytosed viruses. *mBio* *9*, e02314-17.
57. Jaslow, S.L., Gibbs, K.D., Fricke, W.F., Wang, L., Pittman, K.J., Mammel, M.K., Thaden, J.T., Fowler, V.G., Hammer, G.E., Elfenbein, J.R., and Ko, D.C. (2018). Salmonella activation of STAT3 signaling by SarA effector promotes intracellular replication and production of IL-10. *Cell Rep.* *23*, 3525–3536.
58. Grimm, C., Jörs, S., Saldanha, S.A., Obukhov, A.G., Pan, B., Oshima, K., Cuajungco, M.P., Chase, P., Hodder, P., and Heller, S. (2010). Small molecule activators of TRPML3. *Chem. Biol.* *17*, 135–148. <https://doi.org/10.1016/j.chembiol.2009.12.016>.
59. Pujol, C., and Bliska, J.B. (2003). The ability to replicate in macrophages is conserved between *Yersinia pestis* and *Yersinia pseudotuberculosis*. *Infect. Immun.* *71*, 5892–5899. <https://doi.org/10.1128/iai.71.10.5892-5899.2003>.
60. Wang, R.F., and Kushner, S.R. (1991). Construction of versatile low-copy-number vectors for cloning, sequencing and gene expression in *Escherichia coli*. *Gene* *100*, 195–199.
61. Datsenko, K.A., and Wanner, B.L. (2000). One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proc. Natl. Acad. Sci. USA* *97*, 6640–6645. <https://doi.org/10.1073/pnas.120163297>.
62. Cherepanov, P.P., and Wackernagel, W. (1995). Gene disruption in *Escherichia coli*: TcR and KmR cassettes with the option of Flp-catalyzed excision of the antibiotic-resistance determinant. *Gene* *158*, 9–14.
63. Chang, C.C., Chow, C.C., Tellier, L.C., Vattikuti, S., Purcell, S.M., and Lee, J.J. (2015). Second-generation PLINK: rising to the challenge of larger and richer datasets. *Gigascience* *4*, 7. <https://doi.org/10.1186/s13742-015-0047-8>.
64. Chen, S., Zhou, Y., Chen, Y., and Gu, J. (2018). fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* *34*, i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>.
65. Liao, Y., Smyth, G.K., and Shi, W. (2014). featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* *30*, 923–930. <https://doi.org/10.1093/bioinformatics/btt656>.
66. Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol.* *15*, 550. <https://doi.org/10.1186/s13059-014-0550-8>.
67. Dobin, A., Davis, C.A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., and Gingeras, T.R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* *29*, 15–21. <https://doi.org/10.1093/bioinformatics/bts635>.
68. Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A., Paulovich, A., Pomeroy, S.L., Golub, T.R., Lander, E.S., and Mesirov, J.P. (2005). Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc. Natl. Acad. Sci. USA* *102*, 15545–15550. <https://doi.org/10.1073/pnas.0506580102>.
69. Conant, D., Hsiao, T., Rossi, N., Oki, J., Maures, T., Waite, K., Yang, J., Joshi, S., Kelso, R., Holden, K., et al. (2022). Inference of CRISPR edits from sanger trace data. *CRISPR J.* *5*, 123–130. <https://doi.org/10.1089/crispr.2021.0113>.
70. Pruim, R.J., Welch, R.P., Sanna, S., Teslovich, T.M., Chines, P.S., Glied, T.P., Boehnke, M., Abecasis, G.R., and Willer, C.J. (2010). LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics* *26*, 2336–2337. <https://doi.org/10.1093/bioinformatics/btq419>.
71. 1000 Genomes Project Consortium; Auton, A., Brooks, L.D., Durbin, R.M., Garrison, E.P., Kang, H.M., Korbel, J.O., Marchini, J.L., McCarthy, S., McVean, G.A., and Abecasis, G.R. (2015). A global reference for human genetic variation. *Nature* *526*, 68–74. <https://doi.org/10.1038/nature15393>.
72. International HapMap Consortium (2005). A haplotype map of the human genome. *Nature* *437*, 1299–1320. <https://doi.org/10.1038/nature04226>.
73. Purcell, S., Neale, B., Todd-Brown, K., Thomas, L., Ferreira, M.A.R., Bender, D., Maller, J., Sklar, P., de Bakker, P.I.W., Daly, M.J., and Sham, P.C. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* *81*, 559–575. <https://doi.org/10.1086/519795>.
74. Wickham, H. (2016). ggplot2: Elegant Graphics for Data Analysis.
75. Arnold, J.B. (2021). Ggthemes: Extra Themes, Scales and Geoms for 'ggplot2'.
76. 1000 Genomes Project Consortium; Abecasis, G.R., Altshuler, D., Auton, A., Brooks, L.D., Durbin, R.M., Gibbs, R.A., Hurles, M.E., and McVean, G.A. (2010). A map of human genome variation from population-scale sequencing. *Nature* *467*, 1061–1073. <https://doi.org/10.1038/nature09534>.
77. Westermann, A.J., and Vogel, J. (2018). Host-pathogen transcriptomics by dual RNA-seq. In *Bacterial Regulatory RNA: Methods and Protocols*, V. Arluison and C. Valverde, eds. (Springer Sciences), pp. 59–75.
78. Kersey, P.J., Staines, D.M., Lawson, D., Kulesha, E., Derwent, P., Humphrey, J.C., Hughes, D.S.T., Keenan, S., Kerhornou, A., Koscielny, G., et al. (2012). Ensembl Genomes: an integrative resource for genome-scale data from non-vertebrate species. *Nucleic Acids Res.* *40*, D91–D97. <https://doi.org/10.1093/nar/gkr895>.
79. Mootha, V.K., Lindgren, C.M., Eriksson, K.F., Subramanian, A., Sihag, S., Lehar, J., Puigserver, P., Carlsson, E., Ridderstråle, M., Laurila, E., et al. (2003). PGC-1alpha-responsive genes involved in oxidative

- phosphorylation are coordinately downregulated in human diabetes. *Nat. Genet.* 34, 267–273. <https://doi.org/10.1038/ng1180>.
80. Gasnier, B., Zhu, M.X., and Taylor & Francis. Ion and molecule transport in lysosomes. *Methods in Signal Transduction*. First edition. ed.CRC Press
81. Chen, C.C., Butz, E.S., Chao, Y.K., Grishchuk, Y., Becker, L., Heller, S., Slauchaupt, S.A., Biel, M., Wahl-Schott, C., and Grimm, C. (2017). Small molecules for early endosome-specific patch clamping. *Cell Chem. Biol.* 24, 907–916.e4. <https://doi.org/10.1016/j.chembiol.2017.05.025>.
82. Harrell, F.E., Jr. (2021). *Hmisc: Harrell Miscellaneous*.
83. Wickham, H., François, R., Henry, L., and Müller, K. (2022). *Dplyr: A Grammar of Data Manipulation*.

## STAR★METHODS

### KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Bacterial and virus strains</b>		
<i>S. enterica</i> Typhi Ty2 +p67GFP3.1	Dennis Ko <sup>7</sup>	DCK33
<i>S. enterica</i> Typhi Ty2 $\Delta$ ssaT + p67GFP3.1	This paper	DCK723
<i>S. enterica</i> Typhi Ty2 $\Delta$ mgtA $\Delta$ mgtB + p67GFP3.1	This paper	DCK1122
<i>Salmonella enterica</i> Typhi Ty2 with 956bp deletion in <i>phoPQ</i> +p67GFP3.1	Samuel Miller <sup>33</sup>	Ty800 or CS021
<i>S. enterica</i> Typhimurium 14028s +p67GFP3.1	Dennis Ko <sup>5</sup>	DCK22
<i>S. enterica</i> Typhimurium 14028s $\Delta$ mgtA $\Delta$ mgtB + p67GFP3.1	This paper	DCK1121
<i>S. enterica</i> Typhimurium 14028s $\Delta$ mgtA $\Delta$ mgtB + pWSK129	This paper	DCK1132
<i>S. enterica</i> Typhimurium 14028s +pWSK29	Dennis Ko <sup>57</sup>	DCK483
<b>Chemicals, peptides, and recombinant proteins</b>		
Accell siRNA delivery media	Horizon	B-005000
Accell non-targeting #1 (NT1) siRNA	Horizon	D-001910-01
Accell SmartPool <i>MCOLN2</i> siRNA	Horizon	E-021616-00
Accell SmartPool <i>MCOLN3</i> siRNA	Horizon	E-015371-00
Recombinant human IFN- $\gamma$	Peprotech	300-02
Recombinant human IFN- $\beta$	Peprotech	300-02BC
<i>S. Typhimurium</i> S-form LPS	Enzo	ALX-581-011
RNA <sup>later</sup> Solution	ThermoFisher	AM7020
Gentamicin Sulfate	VWR	45000-634
7-aminoactinomycin D (7AAD)	Enzo	ALX-380-283
MgCl <sub>2</sub> Hexahydrate BioReagent	Sigma	M2393
ZnSO <sub>4</sub> Heptahydrate BioReagent	Sigma	Z0251
TaqMan FAM-MGB <i>MCOLN2</i> probe	ThermoFisher	4331182 - Hs00401920
TaqMan FAM-MGB <i>MCOLN3</i> probe	ThermoFisher	4331182 - Hs00962657
Isopropyl $\beta$ -D-1-thiogalactopyranoside (IPTG)	ThermoFisher	15529-019
Hs TRPML2-YFP	Grimm et al. <sup>58</sup>	-
Transfection reagent TurboFect	Thermo Fisher	R0531
Vacuolin	Santa Cruz	sc-216045
PI(3,5)P2	AG Scientific	P-1123
ML2-SA1	Macro Keller and Franz Bracher	-
<b>Critical commercial assays</b>		
mirVana miRNA Isolation Kit	ThermoFisher	AM1560
TURBO DNase	ThermoFisher	AM2238
RNeasy MinElute cleanup Kit	Qiagen	74204
Standard Total RNA Prep with Ribo-Zero Plus	illumina	20037135
iTaq Universal SYBR Green Supermix	BioRad	1725124
RNeasy kit	Qiagen	74106
iScript cDNA Synthesis kit	BioRad	1708891
iTaq Universal Probes Supermix	BioRad	1725134
<b>Deposited data</b>		
Intracellular THP-1 RNA-seq	GEO	GSE222194
Cellular GWAS on Intracellular <i>S. Typhi</i> Replication	Duke Research Data Repository	<a href="https://doi.org/10.7924/r4x92bd76">https://doi.org/10.7924/r4x92bd76</a>

(Continued on next page)

REAGENT or RESOURCE	SOURCE	IDENTIFIER
<b>Continued</b>		
<b>Experimental models: Cell lines</b>		
Human THP-1 Monocytes: WT & <i>MCOLN2</i> <sup>-/-</sup> Pool	Synthego	RRID:CVCL_0006
Human: Lymphoblastoid Cell Lines (LCLs)	Coriell Institute	See Table S1 for all LCL individual identifiers
Human HEK 293	DSMZ	ACC 305
<b>Experimental models: Organisms/strains</b>		
<i>Mus musculus</i> : C57BL/6J <i>Mcoln2</i> <sup>+/-</sup>	Rosa Puertollano	C57BL/6J
<b>Oligonucleotides</b>		
See Table S4 for list of primers.	This study	See Table S4
<b>Recombinant DNA</b>		
p67GFP3.1 (Amp <sup>R</sup> , GFPmut3.1 under P <sub>tac</sub> )	Pujol & Bliska <sup>59</sup>	–
pWSK29 (Amp <sup>R</sup> , very-low copy pSC101 ori)	Wang & Kushner <sup>60</sup>	Addgene #172972
pWSK129 (Kan <sup>R</sup> , very-low copy pSC101 ori)	Wang & Kushner <sup>60</sup>	–
pKD4 (Amp <sup>R</sup> , ts, FRT-Kan <sup>R</sup> -FRT)	Datsenko & Wanner <sup>61</sup>	Addgene #45605
pKD46 (Amp <sup>R</sup> , ts, λ red genes [ <i>exo</i> , <i>bet</i> , <i>gam</i> ] under P <sub>araB</sub> )	Datsenko & Wanner <sup>61</sup>	–
pCP20 (Amp <sup>R</sup> , Cam <sup>R</sup> , ts, <i>Flp</i> )	Cherepanov & Wackernagel <sup>62</sup>	–
<b>Software and algorithms</b>		
GraphPad Prism 9	GraphPad Software	<a href="http://www.graphpad.com">www.graphpad.com</a>
R 4.0.2	R Core Team	<a href="http://www.r-project.org">www.r-project.org</a>
BioRender	BioRender	<a href="http://www.biorender.com">www.biorender.com</a>
PLINK 1.9	Chang et al. <sup>63</sup>	<a href="http://www.cog-genomics.org/plink/">www.cog-genomics.org/plink/</a>
fastp: a FASTQ preprocessor	Chen et al. <sup>64</sup>	<a href="https://github.com/OpenGene/fastp">github.com/OpenGene/fastp</a>
featureCounts tool	Liao et al. <sup>65</sup>	<a href="https://subread.sourceforge.net">subread.sourceforge.net</a>
DESeq2 Bioconductor	Love et al. <sup>66</sup>	<a href="https://bioconductor.org/packages/release/bioc/html/DESeq2.html">bioconductor.org/packages/release/bioc/html/DESeq2.html</a>
STAR RNA-seq alignment tool	Dobin et al. <sup>67</sup>	<a href="https://code.google.com/archive/p/rna-star/">code.google.com/archive/p/rna-star/</a>
GSEA 4.1	Subramanian et al. <sup>68</sup>	<a href="http://www.gsea-msigdb.org">www.gsea-msigdb.org</a>
ICE webtool 2.0	Conant et al. <sup>69</sup>	<a href="http://ice.synthego.com">ice.synthego.com</a>
LocusZoom webtool	Pruim et al. <sup>70</sup>	<a href="http://locuszoom.org">locuszoom.org</a>

## RESOURCE AVAILABILITY

### Lead contact

Further information, as well as plasmids and bacterial strains generated for this study, are available by request from the lead contact, Dennis C. Ko ([dennis.ko@duke.edu](mailto:dennis.ko@duke.edu)).

### Materials availability

Plasmids and bacterial strains, as listed in the [key resources table](#), are available upon request.

### Data and code availability

Intracellular replication data for the 961 LCL samples can be found in [Table S1](#), and GWAS summary statistics are available for download at the Duke Research Data Repository (Duke Research Data Repository: <https://doi.org/10.7924/r4x92bd76>). Intracellular *S. Typhi* RNA-seq data are available in GEO (GEO: GSE222194). The analyses of this data—differential gene expression and gene set enrichment analysis (GSEA)—are available in [Table S2](#). In other replication experiments, the value of each biological replicate is shown as dots on top of bar graphs.

## EXPERIMENTAL MODEL AND SUBJECT DETAILS

### Human cells

Lymphoblastoid cell lines (LCLs; EBV-immortalized B cells) were from the Coriell Institute. *MCOLN2*<sup>-/-</sup> and matched wild-type THP-1 cell pools were generated by Synthego using guide 5'-TTTGGTTTAAAGTAACCAGC-3' (PAM is TGG) to target the start of



*MCOLN2* exon 3. THP-1 knock out pools were confirmed to maintain  $\geq 85\%$  frameshift indels by Sanger sequencing that was analyzed with the inference of CRISPR editing (ICE) webtool v2.0 (<https://ice.synthego.com>) from Synthego.<sup>69</sup> THP-1s and LCLs were maintained at 37°C in a 5% CO<sub>2</sub> atmosphere and were grown in RPMI 1640 media (Gibco #21870) supplemented with 10% heat-inactivated fetal bovine serum (HI-FBS, Gibco #10082), 2 mM L-glutamine (Gibco #25030081), & 100 U/mL Penicillin-Streptomycin (Gibco #15140122). Infection assays were carried out in the same media but without Pen-Strep and phenol red. Cells were verified as mycoplasma free by the Universal Mycoplasma Detection Kit (ATCC #30-1012K).

### Mice

*Mcoln2*<sup>+/-</sup> C57BL/6J mice provided by Dr. Rosa Puertollano and maintained specific pathogen free by Duke DLAR breeding core in groups of 5 or less of the same sex post weaning. Sex of animals is denoted in figure legends. Mice were free fed standard diet (PicoLab Mouse Diet #5058) during infections. Infections were approved by Duke IACUC (protocol #A145-18-06).

### Bacteria

*S. enterica* serovars Typhi strain Ty2, Typhimurium strain 14028s, and derived mutants were grown at 37°C and 250 rpm in high-salt Miller Luria-Bertani (LB) broth (VWR #90003). To quantify intracellular burden during gentamicin protection assays, *Salmonellae* were tagged with inducible GFP using p67GFP3.1,<sup>59</sup> which carries GFP under an IPTG-inducible promoter and is maintained with 100 mg/mL ampicillin. *Salmonella* gene deletion strains were generated by lambda-red recombineering<sup>61</sup> from Ty2 or 14028s using Kan<sup>R</sup> cassettes generated from pKD4 with the primers in Table S4. Gene deletions were confirmed by PCR using indicated primers in Table S4.

## METHOD DETAILS

### Infection (gentamicin-protection) assays

*Salmonella* infection of LCL and THP-1 cells was done as previously described.<sup>5</sup> In brief, overnight stationary cultures in Miller LB were sub-cultured 1:33 and grown for 160 min at 37°C and 250 rpm to reach SPI-1 inducing late-log phase (an OD<sub>600</sub> of 1.7–2.0 for *S. Typhimurium* and 0.8–1.1 for *S. Typhi*).  $1 \times 10^5$  LCL or THP-1 cells were plated at  $1 \times 10^6$  cells/mL in complete RPMI 1 h before infection in 96-well non-TC plates. LCLs were infected at multiplicity of infection (MOI) 30 and THP-1s at MOI 10. To kill the extracellular bacteria, gentamicin was added 1 h post infection (hpi) at 50 mg/mL and then diluted to 15 mg/mL at 2 hpi. In ion repletion experiments, 5 $\mu$ L of filter-sterilized MgCl<sub>2</sub> or ZnSO<sub>4</sub> in DI water, or water only control, was added to 200 $\mu$ L in 96-well plates immediately following gentamicin dilution at 2 hpi. To induce GFP in p67GFP3.1, 1.4 mM IPTG was added 75 min prior to the desired time point.

Invasion, pyroptosis, and initial burden were measured with a Guava EasyCyte Plus high-throughput flow cytometer (Millipore) at 3.5 hpi. Pyroptosis was quantified as the percent staining with 1  $\mu$ g/mL 7AAD (7-aminoactinomycin D; Enzo Life Sciences). Invasion was quantified as the percent GFP<sup>+</sup> & 7AAD<sup>-</sup>. Burden was quantified as median fluorescent intensity (MFI) of living (7AAD<sup>-</sup>) and infected (GFP<sup>+</sup>) cells. Intracellular replication (permissivity) was quantified by re-measuring burden at 24 hpi and taking the ratio of 24 hpi burden over initial 3.5 hpi burden.

### Cellular GWAS

Hi-HoST screening of 961 LCLs from parent-offspring trios for *S. Typhi* intracellular replication occurred in two large sets. In one, *S. Typhi* intracellular replication was one of 79 host-pathogen phenotypes measured as part of the Hi-HoST Phenome Project (H2P2).<sup>6</sup> H2P2 measured replication in 527 LCLs from four population in the 1000 Genomes Project<sup>71</sup>: ESN (Esan in Nigeria), GWD (Gambians in Western Divisions in The Gambia), IBS (Iberian Population in Spain), and KHV (Kinh in Ho Chi Minh City, Vietnam). In this dataset, we determined that replication is a quantitative trait suitable for GWAS due to its inter-individual variation (mean of 1.7-fold with standard deviation of 0.3), high experimental repeatability ( $\sim 75\%$  variance is due to inter-individual variation in two-way ANOVA), and substantial heritability ( $h^2 = 0.33$  with  $p = 0.002$  in parent-offspring regression).<sup>6</sup> To these 527 LCLs, we added previously unpublished data on *S. Typhi* replication from 434 LCLs from four populations in the HapMap project: CEU (Utah residents with ancestry from northern and western Europe), YRI (Yoruba in Ibadan, Nigeria), CHB (Han Chinese in Beijing, China), and JPT (Japanese in Tokyo, Japan).<sup>72</sup> For all 961 LCLs, we used flow cytometry to quantify intracellular bacterial burden as the median fluorescent intensity (MFI) of GFP in infected host cells, which contain viable GFP-tagged *S. Typhi* (see above for details of this fluorescence-based gentamicin protection assay). From these MFI measurements, we calculated intracellular replication or permissivity as the ratio of 24 hpi to 3.5 hpi burden. Each LCL was measured on three sequential passages and the phenotype used for GWAS was calculated as the mean measurement of these three independent assays. Each batch of LCLs measured during Hi-HoST screening was Z score transformed to reduce inter-batch experimental variation:  $Z = (x - \mu_{batch}) / \sigma_{batch}$ .

Genotypes were obtained from HapMap r28 and 1000 Genomes Project Phase 3 with imputation using 1000 Genomes Project Phase 3. Filters included minor allele frequency (MAF) < 0.05, SNP missingness of >0.2 and sample genotype missingness of >0.2, resulting in a total of 8,386,469 SNPs for subsequent analysis. Genome-wide association analysis was carried out using the QFAM-parents approach in PLINK v1.9<sup>24,63,73</sup> with adaptive permutations ranging from 1000 to a maximum of 10<sup>9</sup>. The QFAM approach uses linear regression to test for association while separately permuting between and within family components to control

for family structure. The human genome reference assembly (GRCh37/hg19) was used for all analysis. QQ plots against neutral,  $\chi^2$ , distribution were plotted using quantile-quantile function in R. Local Manhattan plots were generated using LocusZoom<sup>70</sup> webtool (<http://locuszoom.org/>). Linear regression of *Salmonella* replication by rs10873679 genotype was performed and plotted in R using ggplot2<sup>74</sup> & ggthemes<sup>75</sup> packages.

### Human gene expression analyses

RNA-seq gene expression data of 448 LCLs from the 1000 Genomes Project<sup>28</sup> were obtained from the EBI website (<https://www.ebi.ac.uk/gxa/experiments/E-GEUV-1/Downloads>). The rs10873679 genotype data were downloaded from the 1000 genome project.<sup>76</sup> Effects of rs1087369 genotype on *MCOLN2* and *MCOLN3* gene expression in both datasets were tested by linear regression on combined data as well as individual populations and individual sexes. Protein abundance measured by isobaric tag-based quantitative mass-spectroscopy in 95 LCLs from HapMap project were obtained from Wu et al. However, only 33 of the individuals had quantifiable *MCOLN2*. The effect of rs10873679 on *MCOLN2* protein abundance was tested by linear regression in R.

### RNAi experiments and knockdown confirmation

LCLs or THP-1s ( $2.5 \times 10^5$  cells) were washed and re-suspended at 400,000 cells/mL in 500  $\mu$ L of serum-free Accell siRNA delivery media (Horizon #B-005000) in TC-treated 24-well plates and treated for three days with 10 pg/ $\mu$ L of either Dharmacon Accell non-targeting #1 (NT1) (Horizon #D-001910-01) or an Accell SmartPool against human *MCOLN2* (Horizon #E-021616-00) or *MCOLN3* (Horizon #E-015371-00). Prior to infection, cells were washed and plated at 700,000 cells/mL in 100  $\mu$ L complete RPMI media (without antibiotics) in 96-well non-TC plates. Infections were conducted as described above.

For each experiment, knockdown was confirmed by RT-qPCR. Briefly, RNA was extracted from one well not used for infection ( $\sim 5 \times 10^5$  treated cells) for each siRNA condition using RNeasy kit (Qiagen #74106). Then cDNA was reverse transcribed from 500ng of RNA/condition using iScript kit (BioRad #1708891) and quantified by qPCR using iTaq Universal Probes Supermix (BioRad #1725134) and exon-spanning TaqMan FAM-MGB probes (ThermoFisher #4331182; *MCOLN2* is Hs00401920 & *MCOLN3* is Hs00962657) on a QuantStudio 3 thermocycler (ThermoFisher). All qPCR was run in technical triplicate. Mean comparative threshold cycle ( $C_T$ ) value for each transcript was adjusted for input variation by subtracting the mean 18s (*RNA18S5*; ThermoFisher Hs03928990) housekeeping control  $C_T$  from the target gene's  $C_T$  to generate a  $\Delta C_T$ . The  $\Delta\Delta C_T$  for each knockdown was calculated by subtracting target gene  $\Delta C_T$  in siNT1-treated control cells from target gene  $\Delta C_T$  in siTarget-treated cells. Knockdown fold change was then calculated as  $2^{-\Delta\Delta C_T}$ . Mean fold-change knockdown  $\pm$  SEM was reported in figure legends.

### Inducing and measuring *MCOLN2* expression

To measure *MCOLN2* induction,  $5 \times 10^5$  THP-1s in 24-well non-TC treated plates were infected with *S. Typhi* Ty2 at MOI 10 following the above gentamicin-protection assay or stimulated 2 hpi with 500 U/mL (25 ng/mL) recombinant human IFN- $\gamma$  (PeproTech #300-02) and 100 pg/mL well-vortexed *S. Typhimurium* S-form LPS (Enzo #ALX-581-011) or 50 U/mL (5 ng/mL) recombinant human IFN- $\beta$  (PeproTech #300-02BC). At 24hpi, *MCOLN2* expression was measured by RT-qPCR following the same  $\Delta\Delta C_T$  method used to measure knockdown.

### Mouse infections

Litter and sex matched C57BL/6J mice bred from *Mcoln2*<sup>+/-</sup> parents by the Duke DLAR breeding core were infected when 10–18 weeks old with *S. Typhimurium* 14028s sub-cultured 1:33, grown for 160 min to late-log ( $OD_{600}$  1.7–1.9), and then washed twice with sterile PBS. Bacteria were re-suspended in PBS at 10,000/mL based on  $OD_{600}$  and mice were infected via intraperitoneal injection with 100  $\mu$ L of PBS containing 1,000 CFUs. For competitive infections, the initial 1:1 ratio used 500 CFUs of each Amp<sup>R</sup> wild-type (+pWSK29; DCK483) and Kan<sup>R</sup> mutant ( $\Delta$ *mg*A $\Delta$ *mg*B + pWSK129; DCK1132) *S. Typhimurium*. All inoculums were verified by plating for CFUs. All mice were monitored twice daily for morbidity. Spleens were harvested 4 dpi, homogenized by bead beating with ZrO beads (GlenMills #7305-000031) with a Bead Ruptor 12 (Omni #19-050A), and a serial dilution was plated for CFUs on LB + Amp (100  $\mu$ g/mL) or Kan (50  $\mu$ g/mL). Competitive index was calculated as ratio of Kan/Amp CFUs.

### Fluorescence-activated cell sorting (FACS)

For cell sorting RNA-seq samples, 20 million THP-1 monocytes of each *MCOLN2* genotype were plated into 24-well non-TC plates (500,000 cells per 0.5 mL RPMI per well) and infected with *S. Typhi* at MOI10. The remaining late-log *S. Typhi* inoculum was washed with PBS and fixed with 100  $\mu$ L of RNA<sup>later</sup> Solution (ThermoFisher #AM7020) for 10 min at room temperature and then frozen for later RNA extraction. Following 2 h of IPTG induction, monocytes were spun down at 16 hpi and re-suspended at 10 million cells/mL in 2 mL of RPMI containing 15  $\mu$ g/mL gentamicin and 1  $\mu$ g/mL 7AAD. Two wells containing one million uninfected THP-1 monocytes of each genotype were washed with PBS and fixed in 1mL RNA<sup>later</sup> for use in the control.

Live monocytes were analyzed and sorted by the Duke Human Vaccine Institute (DHVI) flow cytometry shared resource using a FACSAria II (BD Biosciences) at 70 psi with at 70  $\mu$ m nozzle. One million infected (GFP<sup>+</sup>) and living (7AAD<sup>-</sup>) cells were sorted into 1 mL of RNA<sup>later</sup> for immediate fixation and held at 4°C in a chilled collection tube rack. Doublets were excluded by FSC and SSC gating and a purity mask was applied to exclude droplets containing GFP<sup>+</sup> and GFP<sup>-</sup> events.

### S. Typhi infected THP-1 RNA extraction

After sorting, RNA was immediately isolated from collected cells using mirVana miRNA Isolation Kit's total RNA protocol (ThermoFisher #AM1560). Prior to extraction, samples in RNAwater were diluted 3x with PBS, spun down at 5,000 xg, and aspirated to remove RNAwater. After resuspending cells in the kit's L/B buffer, samples were vortexed for 60 s to ensure lysis of *Salmonella*. Following total RNA extraction, gDNA was removed with 4 U TURBO DNase (ThermoFisher #AM2238) per  $\mu\text{g}$  of RNA and then purified with RNeasy MinElute cleanup kit (Qiagen #74204). In the gDNA-free RNA, the relative human to bacterial mRNA ratio was determined by RT-qPCR measurement of human *ACTB* and *S. Typhi rpoD*. In brief, 150ng of RNA was reverse transcribed with iScript cDNA synthesis kit (Bio-Rad #1708891) and 1:5 dilution of this cDNA was analyzed using iTaq Universal SYBR Green Supermix (Bio-Rad #1725124) and primers listed in Table S4 on a QuantStudio 3 System (ThermoFisher). This ratio was used to combine uninfected THP-1 mRNA and late-log *S. Typhi* inoculum mRNA for the control samples following Westermann & Vogel's approach.<sup>77</sup>

### THP-1 and intracellular S. Typhi Dual RNA-seq

RNA was isolated from three independent experiments and submitted to the Duke Sequencing and Genomic Technologies (SGT) Shared Resource for cDNA library preparation with Illumina Standard Total RNA Prep with Ribo-Zero Plus (Illumina #20037135). These rRNA-depleted libraries were sequenced on Illumina NovaSeq 6000 S prime flow cell with 100 bp paired-end reads.

RNA-seq data were processed using the fastp toolkit<sup>64</sup> to trim low-quality bases and Illumina sequencing adapters from the 3' end of the reads. Only reads that were  $\geq 20\text{nt}$  after trimming were kept for further analysis. Reads were mapped to a custom genome reference combining the GRCh38v93 version of the human genome and transcriptome<sup>78</sup> with *S. enterica* serovar Typhi strain Ty2 ASM754v1 genome and transcriptome using the STAR RNA-seq alignment tool.<sup>67</sup> Reads were kept for subsequent analysis if they mapped to a single genomic location. Gene counts were compiled using the featureCounts tool.<sup>65</sup> Only genes that had at least 10 reads in any given library were used in subsequent analysis. Normalization and differential expression within each species was carried out using the DESeq25 Bioconductor<sup>66</sup> package with the R statistical programming environment. The FDR was calculated to control for multiple hypothesis testing.

### S. Typhi gene set enrichment analysis (GSEA)

Intracellular *S. Typhi* RNA-seq results were converted into a ranked gene list by multiplying the  $\log_2(p)$  by the sign of the  $\log_2$  fold-change (expression inside KO/WT THP-1). Fifteen *S. Typhi* gene sets related to divalent cation transport or virulence were generated, as shown in Table S1, and analyzed using GSEA v4.1.<sup>68,79</sup>

### Endolysosomal patch-clamp experiments

The protocol of whole-endolysosome recordings have been described previously in detail.<sup>47,80</sup> HEK-293 cells were plated onto poly-L-lysine (Sigma)-coated glass coverslips. Human TRPML2 WT was transiently transfected into HEK-293 cells using TurboFect Transfection Reagent (Thermo Fisher Scientific) for 16–24 h. Cells were treated with vacuolin-1, a lipid-soluble polycyclic triazine that selectively enlarges endolysosomes homotypically (HEK293 cells, 1  $\mu\text{M}$  overnight) up to 2–5  $\mu\text{m}$  (capacitance =  $0.39 \pm 0.01$  pF,  $n = 12$  vacuoles). Vacuolin-1 were washed out before patch-clamp experimentation. Electrophysiological data were recorded using EPC-10 patch-clamp amplifier (HEKA, Lambrecht, Germany), Axonpatch 200B (Molecular Devices), PatchMaster acquisition software (HEKA) and pClamp v10 software (Molecular Devices). Ramp protocol (–100 mV to +100 mV in 500 ms, holding potential = 0 mV). The current amplitudes at –100 mV were extracted from individual ramp recordings. Data were digitized at 40 kHz and filtered at 2.8 kHz. The compensation of capacitive transients and liquid junction potential were corrected as described.<sup>81</sup> For the application of 10  $\mu\text{M}$  diC8-PI(3,5)P<sub>2</sub> (AG Scientific) or 30  $\mu\text{M}$  ML2-SA1 in the bath solutions, the perfusion system and direct bath application were performed. ML2-SA1 were kindly provided by Macro Keller and Franz Bracher.<sup>46</sup> The cytoplasmic solution (bath) contained 150 mM NMDG and 10 mM HEPES (pH 7.2). Luminal solution (pipette) contained 105 MgCl<sub>2</sub>, 5 mM HEPES, and 5 mM MES (2-(N-Morpholino)-ethane sulfonic acid) (pH 7.2). All statistical analysis was done using Origin9 software.

### QUANTIFICATION AND STATISTICAL ANALYSIS

Descriptive statistics were performed with GraphPad Prism v9 (GraphPad Software, US) or R v4.0.2 (R Core Team) using Hmisc<sup>82</sup> & dplyr<sup>83</sup> packages. All replication ratios were  $\log_2$ -transformed or z-scored by batch before analysis. The size of each study or number of replicates, along with the statistical tests performed can be found in figure legends. Unless otherwise indicated, all datasets passed normality tests indicating no significant deviation from a Gaussian distribution. *In vitro* inter-experimental variability was removed prior to data visualization or statistical analysis by making experimental means equal to the grand mean by multiplying all values within each experiment by a normalization constant. These constants were calculated by dividing the mean of all experiments by mean of each specific experiment. Bar graphs represented the mean  $\pm$  SEM (standard error of mean), unless otherwise noted. If an outlier was removed, it is noted in the figure legend along with the original value and the test used to exclude it.

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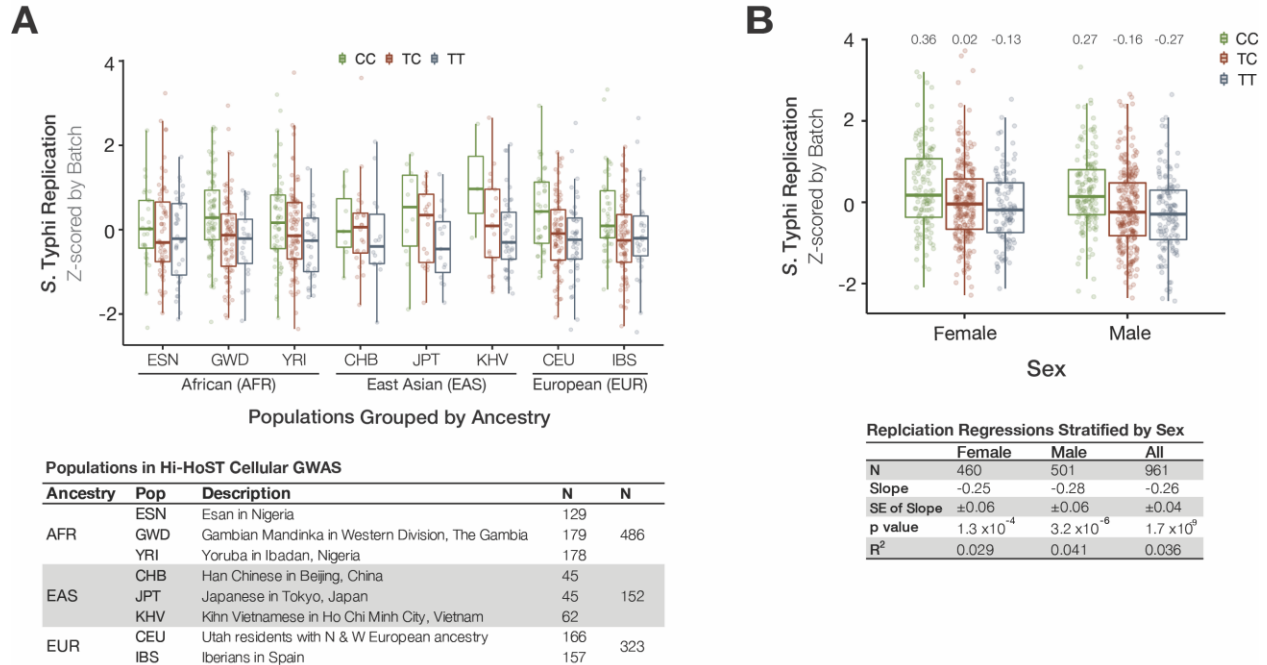
## Supplemental information

### Human variation impacting

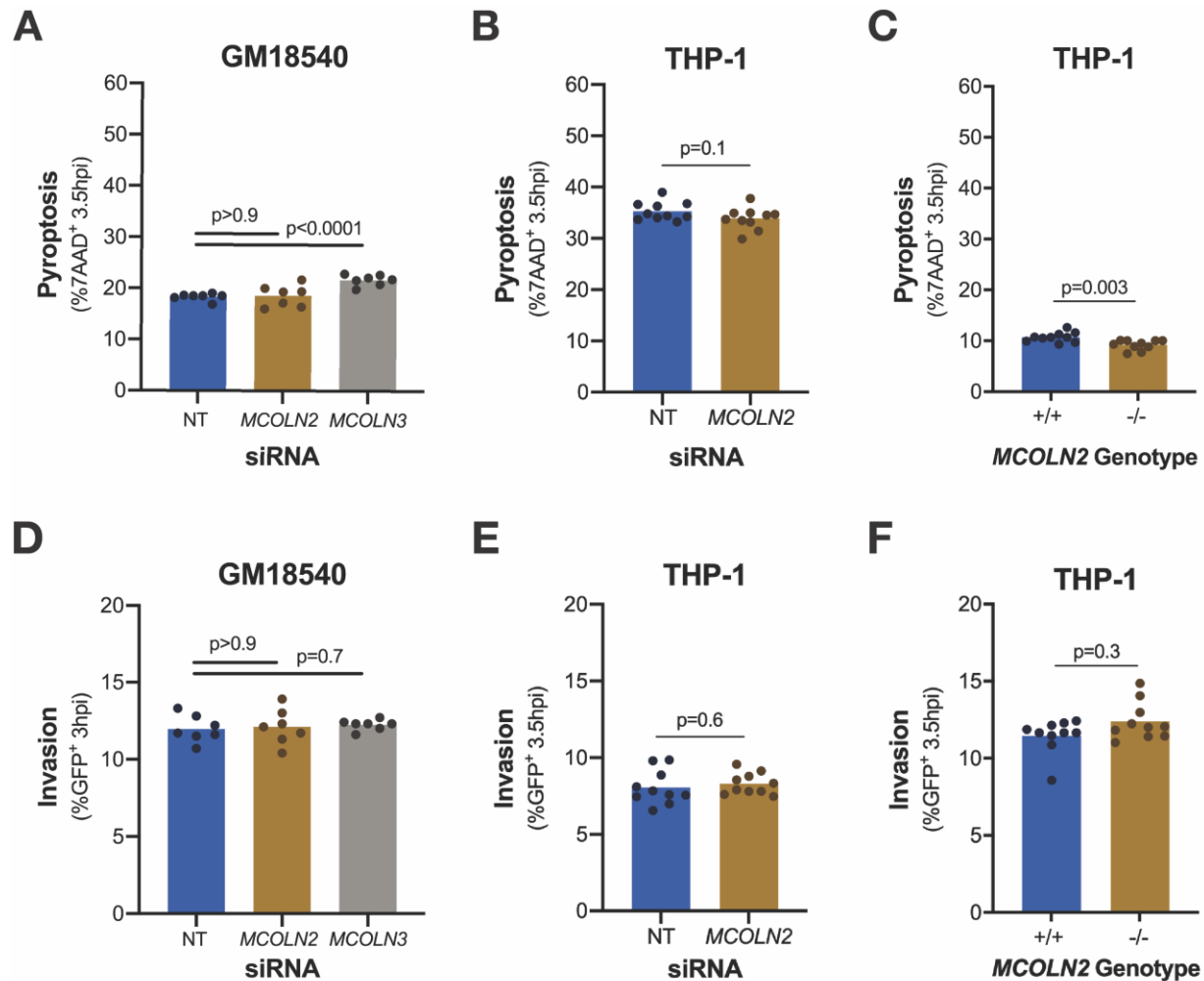
***MCOLN2* restricts *Salmonella* Typhi**

**replication by magnesium deprivation**

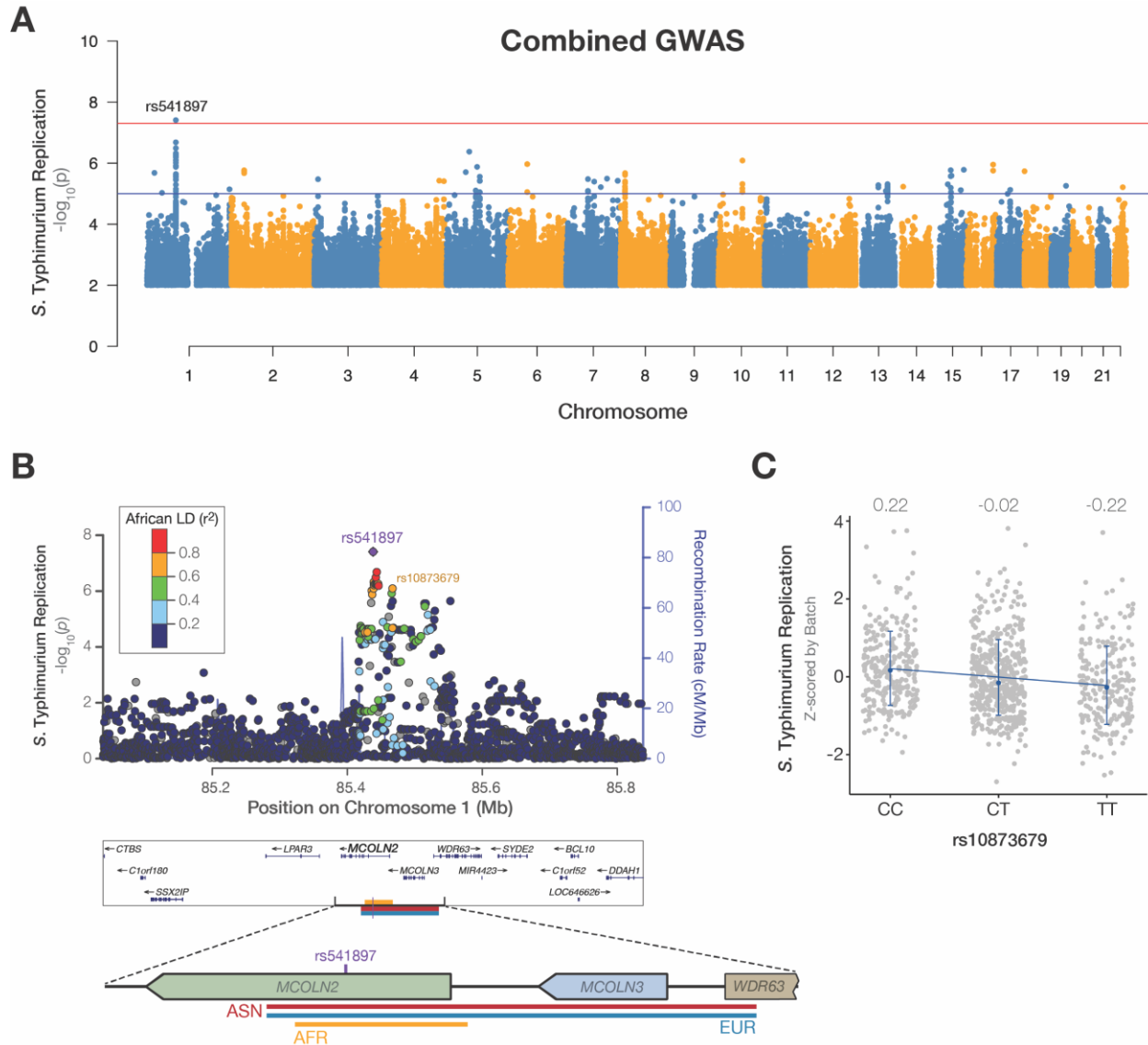
**Kyle D. Gibbs, Liuyang Wang, Zhuo Yang, Caroline E. Anderson, Jeffrey S. Bourgeois, Yanlu Cao, Margaret R. Gaggioli, Martin Biel, Rosa Puertollano, Cheng-Chang Chen, and Dennis C. Ko**



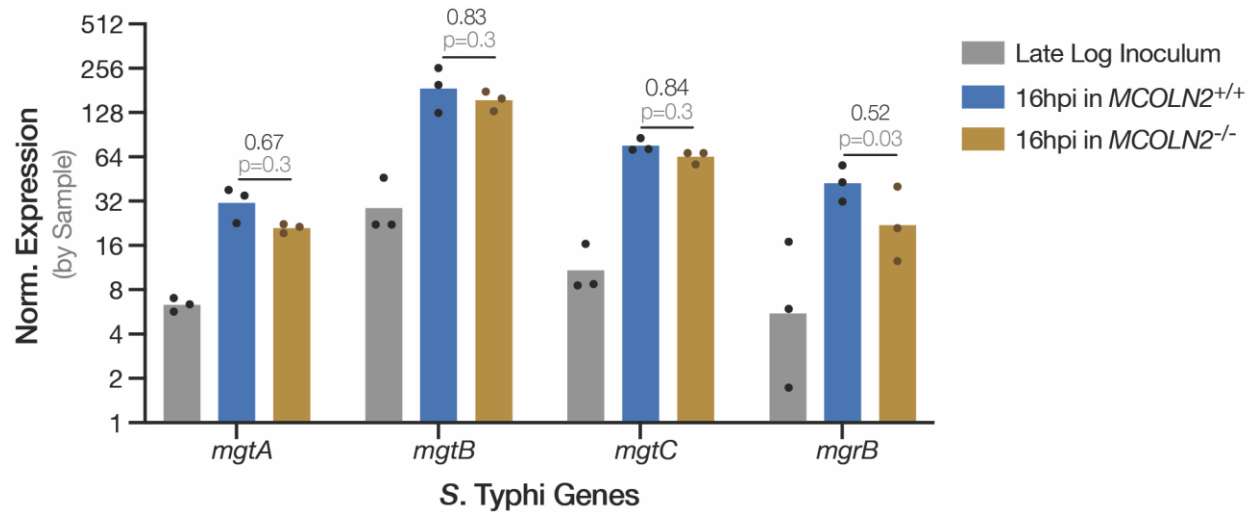
**Figure S1. The rs10873679 C-allele associates with more *S. Typhi* replication across all sampled populations and in both sexes, related to Figure 1. (A) Increased z-scored *S. Typhi* replication associated with the C-allele in all populations. Individual population regressions slopes range from -0.12 in ESN to -0.40 in GWD. Table below lists number of LCLs from each population used in cellular GWAS. (B) The rs10873679 C-allele associated with more z-scored *S. Typhi* replication in both sexes. Linear regressions are listed in the table. A two-way ANOVA with genotype and sex finds no significant interaction between sex & *MCOLN2* genotype ( $p = 0.8$ ) despite both individual factors being a significant source of variation (genotype  $p=1.9 \times 10^{-9}$  & sex  $p=0.02$ ).**



**Figure S2. Neither *MCOLN2* knockdown nor knockout affect pyroptosis or invasion during *S. Typhi* infection in Figure 2E-G.** (A–C) Pyroptosis is the percent of cells dead (7AAD<sup>+</sup>) at 3.5 hpi. (D–E) Invasion is the percent of living (7AAD<sup>-</sup>) and infected (GFP<sup>+</sup>) cells at 3.5 hpi. LCL (GM18540) infection in A & D are nine replicates from three experiments from Figure 2E. THP-1 infection in B, C, E, & F are ten replicates from two experiments presented in Figure 2F & G. In A & F, the p values are from Mann-Whitney U tests. In B-E, p values are from unpaired *t* tests. While wild-type and knockout cells significantly differ in C, the effect size is only 0.5% respectively.



**Figure S3. Increased *S. Typhimurium* replication also associates with the rs10873679 C- allele, related to Figure 2H.** (A) The rs541897 locus association with z-scored *S. Typhimurium* replication ( $p = 3.9 \times 10^{-8}$ ) passes genome-wide significance threshold ( $5 \times 10^{-8}$ ; red line). (B) A local Manhattan plot of the *S. Typhimurium* replication-associated locus on chromosome 1 (hg19 build) shows that the *S. Typhimurium* lead SNP, rs541897, is in the same locus as the *S. Typhi* lead SNP, rs10873679. The *S. Typhi* and *S. Typhimurium* lead SNPs are linked (LD  $r^2 = 0.71$  in African ancestry populations). Dots for each SNP are color coded by African (AFR) linkage disequilibrium (LD;  $r^2$ ) from 1000 genomes Nov 2014 release. A 164 kb zoom in on the *MCOLN2/3* region indicates regions well-linked ( $r^2 > 0.6$ ) with rs541897 by continental ancestry: African with a 41 kb orange bar, European (EUR) with a 116 kb blue bar, & Asian (ASN) with a 116 kb red bar. (C) The rs10873679 C-allele associates with more *S. Typhimurium* replication. The slope of a linear regression predicting replication from genotype is significantly different from zero ( $\beta = -0.22 \pm 0.04$ ;  $p = 3.5 \times 10^{-6}$ ). Mean replication of each genotype is listed above their respective dot plot. Bars are mean  $\pm$  SD.



**Figure S4. The increased expression of  $Mg^{2+}$ -regulated *S. Typhi* genes inside THP-1 monocytes is consistently decreased in *MCOLN2* knockouts, same data underlying  $\log_2$ [Fold Change] in Figure 3D.** Three replicates are from three experiments. Bars are geometric mean. Inoculum RNA was mixed with RNA from uninfected THP-1 cells of each genotype, so each late log replicate is the average of two technical (sequencing) replicates. Ratios (black text) are geometric mean from expression of bacteria replicating inside *MCOLN2* knockout over bacteria replicating in wild-type THP-1 cells. P values (grey) are from paired t tests corrected for multiple testing by Holm-Šidák method.



**Table S3.** *Salmonella enterica* ser. Typhi strain Ty2 gene sets used in GSEA. Related to Figures 3C and 3D.

Gene Sets	N	Genes
Housekeeping	9	<i>rpoD aroC dnaN hemD hisD sucA thrA dnaK rpoA</i>
SPI-1	24	<i>sicP sptP iacP prgH invA sigE invF spaM prgl invF sipA sipB sipC sipD spaS spaR spaP spaO hilA hilD spaK spaJ spaI spaH</i>
SPI-2	23	<i>spiC spiA ssaD ssaE sseA sseB sscA sseC sseD sseE sscB sseF sseG ssaG ssal ssaJ ssaK ssaL ssaR ssaR ssaS ssaT ssaU</i>
PhoP Regulon	19	<i>pmrD mgtA phoN phoP phoQ mgtB virK ybjX mgrB pagP pagD iraP pagC mgtC pipD pagK ugtL pgtE spiC</i>
Magnesium Influx	4	<i>mgtA mgtB mgtS mgrB</i>
PmrA Regulon	11	<i>pmrC pmrA pmrB naxD arnB arnC arnA arnD arnT arnE arnF</i>
Zinc Efflux	4	<i>fieF zntA zntB zntB</i>
Zinc Influx	4	<i>zupT znuA znuB znuC</i>
Ferrous Iron Efflux	2	<i>fieF iceT</i>
Ferrous Iron Influx	3	<i>feoA feoB feoC</i>
Manganese Influx	7	<i>mntH sitA sitB sitC sitD mntR mntS</i>
Copper Response	8	<i>copA cueR cueO cueP scsA scsB scsC scsD</i>
MFS Efflux	5	<i>mdtH mdtM mdtG emrD mdfA</i>
ToiC Efflux	5	<i>macB tolC acrA acrE marA</i>
RpoE Regulon	5	<i>rpoE rseA rseB rseC</i>

**Table S4.** Oligonucleotides grouped by function in study. Related to STAR Methods.

<b>Lambda-Red Recombination</b>				
Target Gene	Gene ID	Primer Designations	Fwd for Kan <sup>R</sup> Cassette Generation	Rvr for Kan <sup>R</sup> Cassette Generation
<i>mgtA</i>	t4491	DK972 & DK973	TATAATCCGCGGCGCAATTATTTACTTACCGGAGGCGAC gtgtaggctggagctgcttc	TCGGGGATTAAGCACGCTGGCGAATCCCGACGAAAGTGT catatgaatatcctccttag
<i>mgtB</i>	t3755	DK974 & DK975	ATATGCAGGAAACACTACACCTTAATTTGGGGATTCATC gtgtaggctggagctgcttc	TATCGGGTGAGCGATTTCATCTGGGGGATCCTCAAACATTA catatgaatatcctccttag
<i>ssaT</i>	t1289	DK3 & DK433	taccocggcagataatgttaacgaattggagagcatggttga gtgtaggctggagctgcttc	TCACGTAATTTCTTTCTGTAGGCTGTCTGTTTTCTCGC catatgaatatcctccttag
<b>Deletion Confirmation</b>				
Target Gene(s)	Gene ID	Primer Designations	Fwd	Rvr
<i>phoPQ</i>	t1689 & t1690	DK1008 & DK1009	CATGACGCCGCAAAATTATATC	GAAAGTCGGCCAGTTAAGA
<i>ssaT</i>	t1289	DK434 & DK435	CGGTAGTTGGTGTCACTCGTAAG	AGCGCAATCAGCTGAAATAATG
<i>mgtA</i>	t4491	DK980 & DK981	CGTGACGCTGATGGTGATAAA	ACATCTCCTCTCTCGTTCTG
<i>mgtB</i>	t3755	DK982 & DK983	CAGGCGTATAAGGAGGGAATG	ACACCAACAGGCTAATCAGTAA
<i>MCOLN2</i>	ENSG00000153898	DK859 & DK860	CTGGGGTAATTTCCAAAAGCAGT	AGGTTATTCTTCTGTGGACTTGT
<b>SYBR RT-qPCR</b>				
Target Gene(s)	Gene ID	Primer Designations	Fwd	Rvr
<i>ACTB</i>	ENSG00000075624	DK880 & DK881	CCTGTACGCCAACACAGTGC	ATACTCTGCTTGCTGATCC
<i>rpoD</i>	t3131	DK628 & DK629	GAAATGGCACTGTTGAAC TG	CAGATAGGTAATGGCTTCCGG