



HAL
open science

Host resistance factor SLC11A1 restricts Salmonella growth through magnesium deprivation

Olivier Cunrath, Dirk Bumann

► **To cite this version:**

Olivier Cunrath, Dirk Bumann. Host resistance factor SLC11A1 restricts Salmonella growth through magnesium deprivation. *Science*, 2019, 366 (6468), pp.995-999. 10.1126/science.aax7898 . hal-04308484

HAL Id: hal-04308484

<https://hal.science/hal-04308484v1>

Submitted on 15 Oct 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Title:** Host-resistance factor SLC11A1 restricts *Salmonella* growth through
2 magnesium deprivation

3
4
5 **Authors:** Olivier Cunrath¹, Dirk Bumann^{1*}

6 **Affiliations:** Biozentrum, University of Basel, CH-4056 Basel, Switzerland

7
8 *Correspondence to: dirk.bumann@unibas.ch.

9
10 **Abstract:** The pleiotropic host-resistance factor SLC11A1 (NRAMP1) defends against diverse
11 intracellular pathogens in mammals by as yet unknown mechanisms. We compared *Salmonella* infection
12 of coisogenic mice with different *SLC11A1* alleles. SLC11A1 reduced *Salmonella* replication and
13 triggered upregulation of uptake systems for divalent metal cations but no other stress responses.
14 SLC11A1 modestly diminished iron availability and acutely restricted *Salmonella* access to magnesium.
15 Growth of *Salmonella* cells in presence of SLC11A1 was highly heterogeneous and inversely correlated
16 with expression of the crucial magnesium transporter gene *mgtB*. We observed superimposable single-cell
17 patterns in mice lacking SLC11A1 when we restricted *Salmonella* access to magnesium by impairing its
18 uptake capabilities. Together, these findings identify deprivation of the main group metal magnesium as
19 main resistance mechanism of SLC11A1 against *Salmonella*.

20
21 **One sentence summary:** A macrophage protein limits metal availability

22

23 **Main text:** Solute carrier family 11, member 1 (SLC11A1; also called natural resistance-associated
24 macrophage protein 1, NRAMPI) is a host-resistance factor that controls susceptibility to the intracellular
25 pathogens *Salmonella*, *Mycobacteria*, and *Leishmania (L)*. SLC11A1 transports Fe^{2+} , Mn^{2+} , and Co^{2+} out
26 of phagosomes, and may deprive vacuolar pathogens of these essential micronutrients. In addition,
27 SLC11A1 modulates phagosome maturation; pro-inflammatory cytokines and activation of innate
28 lymphocytes; generation of nitric oxide, reactive oxygen species, and lipocalin 2; as well as mammalian
29 iron homeostasis (Fig. S1). The main mechanisms of SLC11A1-mediated resistance are still unclear, and
30 metal starvation in the phagosome might be secondary to the effects of inflammatory responses and
31 altered host iron homeostasis (*L*).

32 We generated coisogenic mice carrying the D169 null allele (*SLC11A1^s*, s) or the functional G169
33 allele (*SLC11A1^r*, r) in the C57BL/6 background. After infection with *Salmonella*, heterozygous (r/s) and
34 particularly homozygous (r/r) mice showed delayed symptoms and reduced *Salmonella* colonization of
35 spleen compared to (s/s) mice (Fig. 1A; Fig. S2a,b). (r/s) and (r/r) mice still succumbed to the disease
36 from day 7 post-infection in contrast to 129S2/SvPasCrl mice which survived *Salmonella* infection
37 indicating contributions of additional resistance loci (2). *SLC11A1^r* reduced *Salmonella* replication as
38 measured with the growth reporter TIMER^{bac} (3), from $0.14 \pm 0.02 \text{ h}^{-1}$ in (s/s) mice to $0.08 \pm 0.02 \text{ h}^{-1}$ in
39 (r/s) mice and $0.065 \pm 0.01 \text{ h}^{-1}$ in (r/r) mice, thus approximating division rates in highly resistant
40 129S2/SvPasCrl mice (Fig. 1B,C; Fig. S2c,d). Slow replication was maintained through day 6 post-
41 infection, and fully explained the reduced *Salmonella* loads in (r/s) and (r/r) mice, but not in
42 129S2/SvPasCrl mice (Fig. 1A,C,D). SLC11A1 thus regulated *Salmonella* replication (4), while
43 additional loci in 129S2/SvPasCrl mice enhanced killing of *Salmonella*.

44 We sorted *Salmonella* from spleen and determined their protein profiles. We analyzed (s/s) mice
45 at day 4 as they succumbed to infection from day 5. For (r/s) and (r/r) mice, we had to wait until day 6, or
46 use higher inocula, to obtain sufficient *Salmonella* material. SLC11A1 had similar impact on *Salmonella*
47 growth at day 4 and 6 after infection (Fig. 1C), and after low- and high-dose infection (Fig. S1e).

48 Comparison of 1833 *Salmonella* proteins revealed increased abundance of uptake systems for metal
49 cations Mg^{2+} , Zn^{2+} , $Fe^{2+/3+}$, and Mn^{2+} in (r/s) and particularly (r/r) mice (Fig. 2A, colored circles).
50 Ribosome subunits were less abundant in (r/s) and (r/r) mice (Fig. 2A, black circles), consistent with
51 slower *Salmonella* replication (Fig. 1C). Otherwise, *Salmonella* from the different mouse genotypes were
52 rather similar (Fig. S3a). We did not observe signs for differential oxidative or nitrosative stress in
53 agreement with the activities of respective *Salmonella* reporter strains (Fig. 2B; Fig. 3b) (5) and genetic
54 evidence against a role of these stresses in SLC11A1-mediated resistance (6). Major virulence systems
55 and metabolic and cell envelope stress regulons were also unaltered (Fig. S3a). Finally, comparison to
56 proteomes of slow- and fast-growing *Salmonella* subsets from BALB/c (s/s) mice (3) (Fig. S3c) indicated
57 fundamentally different mechanisms of *Salmonella* growth-control in presence or absence of SLC11A1.
58 Taken together, SLC11A1 specifically induced *Salmonella* metal uptake systems but no other stress
59 responses.

60 *Salmonella* encodes transporters with different affinities for divalent metal cations (Fig. 3A, table
61 S1). We compared the fitness of *Salmonella* strains with single and multiple gene deletions (detailed
62 description in Supplementary Text) in littermates from (r/s) x (s/s) crosses. We analyzed (s/s) mice after
63 four days, and (r/s) after six days, to match the number of *Salmonella in-vivo* divisions (Fig. 3B; Fig. S4).
64 For relevant mutants, we additionally analyzed (r/r) mice at day 4 and 6 (Fig. 3C; Fig. S4).

65 The quadruple iron-uptake mutant “c” had compromised fitness in (s/s) mice, a stronger defect in
66 (r/s) mice, and almost no fitness in (r/r) mice. Available iron levels *in vivo* seemed to be generally
67 insufficient to meet the high requirements of this mutant (30 μ M iron *in vitro*, Fig. 3D), and SLC11A1
68 further restricted iron access (7). By contrast, the double iron-uptake mutant “b” retained full fitness in all
69 three mouse genotypes indicating sufficient iron supply to meet the lower requirements of this mutant (3
70 μ M iron *in vitro*; Fig. 3D). Wild-type *Salmonella* required even less iron and should thus not be limited
71 by iron starvation either. SLC11A1 thus reduced iron availability but this was insufficient for limiting
72 replication of wild-type *Salmonella*.

73 Mutant “e” lacking the high-affinity zinc ABC transporter had poor fitness in both (s/s) and (r/s)
74 mice indicating limited zinc supply (8). By contrast, mutant “d” with a partial zinc uptake defect (9) had a
75 slight, but significant fitness defects in (s/s) and (r/s) but not (r/r) mice indicating nearly sufficient access
76 to zinc in all three mouse genotypes. Wild-type *Salmonella* with full zinc uptake capabilities would thus
77 not be limited by zinc limitation either. Alternatively, low zinc concentration might activate host
78 endosomal hydrolases such as cathepsins that could damage *Salmonella* cells (10). However, cathepsin
79 activities did not co-localize with *Salmonella* in (s/s) and (r/s) mice (Fig. S5) (11). Mutants lacking uptake
80 systems for manganese (“f”) or cobalt (“h”), or exporters for manganese (“g”) or copper (“i”) had no
81 differential fitness defects arguing against contributions of these metals to SLC11A1-mediated resistance.
82 Although transition metals might become more important at later disease stages with distinct host-control
83 mechanisms (12) and decreasing levels of non-heme iron in spleen (13, 14), particularly in genetic
84 backgrounds with lower serum iron levels (15), our data indicate that transition metal starvation is not
85 required for SLC11A1-mediated resistance.

86 We also analyzed *Salmonella* mutants with defects in uptake systems for the main group metal
87 magnesium, since its transporter MgtB was highly upregulated in presence of SLC11A1 (Fig. 2A). The
88 double mutant “l” with inactivated MgtA and MgtB had severe fitness defects in all three mouse
89 genotypes (16) suggesting that (i) the third magnesium transporter CorA did not contribute to magnesium
90 uptake *in vivo* (16), and (ii) magnesium availability *in vivo* was insufficient to meet the requirements of
91 this mutant. Mutant “l” might also suffer from dose-dependent iron accumulation (17), although this
92 should cause the most severe fitness defects in (s/s) mice with the highest iron supply (see above), which
93 was inconsistent with our observations. Mutant “j” with inactivated MgtA retained full fitness in all
94 mouse genotypes (18), while mutant “k” with inactivated MgtB had full fitness in (s/s) mice, but
95 compromised fitness in (r/s) and (r/r) mice (Fig. 3B,D; Fig. S3) suggesting sufficient magnesium
96 availability only in (s/s) mice. An independently constructed $\Delta mgtB$ mutant had similar differential fitness
97 phenotypes. SLC11A1 thus caused a specific requirement for MgtB (apparent K_m of 6 μM for Mg^{2+} (19)),

98 whereas MgtA with slightly lower affinity (apparent K_m 29 μ M (19)) was dispensable in the presence of
99 MgtB, and compensated only partially for its absence. Attenuation of *Salmonella* Δ *mgtB* in genetically
100 resistant C3H/HeN mice (20), but not in susceptible BALB/c mice (18), has previously been reported.
101 Our data link this phenotype to SLC11A1 instead of the many other allelic differences between C3H/HeN
102 and BALB/c mice and suggest that SLC11A1 critically limits magnesium availability for *Salmonella*.

103 To determine how *mgtB* expression relates to the remarkably heterogeneous replication of
104 *Salmonella* single-cells (Fig. 1B), we combined a *timer*^{bac} expression cassette reporting single-cell
105 division rates (3), with a transcriptional fusion of the $P_{mgtCBRcigR}$ promoter driving *mgtB* expression to *bfp*
106 (Fig. S6a-c). *Salmonella mgtB* expression and division rate were inversely correlated in (r/s) and (r/r)
107 mice, but not in (s/s) mice (Fig. 4A,B). In presence of SLC11A1, poorly replicating *Salmonella* cells thus
108 maximized Mg^{2+} uptake through the crucial transporter MgtB, possibly in order to compensate for
109 magnesium limitation. To test this hypothesis, we deprived *Salmonella* of magnesium by an independent
110 mechanism, and determined the effects in susceptible (s/s) mice that normally provide sufficient
111 magnesium for *Salmonella* growth (see above). Consistent with our fitness data (Fig. 3B), *Salmonella*
112 *mgtB* D379A had similar single-cell division rates as wild-type (Fig. 4C). Additional inactivation of
113 MgtA abolished replication and triggered exceedingly high *mgtB* expression suggesting that *Salmonella*
114 cells tried to maximize their Mg^{2+} uptake capabilities (Fig. 4A,C). For less severe growth restriction, we
115 preserved functional MgtA but reduced its abundance with an inefficient ribosomal binding site or an
116 unconventional initiation codon. While *Salmonella mgtB* D379A RBS*-*mgtA* had slightly reduced
117 division rates, *Salmonella mgtB* D379A AUA-*mgtA* had substantially reduced division rates in (s/s) mice
118 approximating those of wild-type *Salmonella* in (r/s) and (r/r) mice (Fig. 4A,C). By impairing magnesium
119 uptake, we could thus achieve similar levels of growth restriction as imposed by SLC11A1.
120 Astonishingly, this was also associated with superimposable single-cell *mgtB* expression (Fig. 4A,C).
121 Individual *Salmonella* cells thus showed qualitatively and quantitatively equivalent responses to
122 magnesium deprivation because of defective uptake, as to growth limitation by SLC11A1. By contrast,

123 expression of the critical zinc-transporter *znuA* poorly correlated with *Salmonella* division rate in all three
124 mouse genotypes (Fig. 4B) and showed discordant effects of limiting zinc uptake (Fig. S7a,b), consistent
125 with mutant fitness data arguing against relevant zinc deprivation (Fig. 3A,B). Together, these data
126 identify magnesium deprivation as main resistance mechanisms of SLC11A1.

127 These findings were initially surprising as SLC11A1 has been shown to transport Fe^{2+} , Mn^{2+} , and
128 Co^{2+} (1). However, the apparent affinities for these metals are in the low micromolar range (21), and it is
129 unclear how SLC11A1 should outcompete corresponding *Salmonella* transporters with 10 to 1,000 fold
130 better affinities (table S1). On the other hand, Mg^{2+} is the most abundant divalent cation in mammalian
131 cells with free concentrations around 0.5 - 1 mM in the cytosol as well as in *Salmonella*-containing
132 vacuoles early after phagocytosis (22-24). Bacterial cells require around 20 mM Mg^{2+} in their cytosol (25)
133 and proliferating *Salmonella* may quickly exhaust available Mg^{2+} in the vacuole, as leukocyte membranes
134 represent tight barriers for Mg^{2+} (22, 23). Indeed, Mg^{2+} transport becomes relevant for *Salmonella* growth
135 several hours after phagocytosis (26), but vacuolar Mg^{2+} concentration at such late time points are
136 currently inaccessible (24). SLC11A1 could restrict *Salmonella* access to Mg^{2+} by modulating fusion of
137 *Salmonella*-containing vacuoles with Mg^{2+} -containing vesicles (27), or SLC11A1 might directly transport
138 Mg^{2+} similar to some bacterial SLC11A1 orthologs (28, 29). Addressing these issues will require
139 overcoming challenges in purifying active SLC11A1 and establishment of Mg^{2+} transport assays without
140 unavailable radioisotope ^{28}Mg . More generally, this study demonstrates how integrated single-cell
141 analysis of pathogens can elucidate relevant mechanisms of pleiotropic host-resistance factors. This
142 strategy may be applicable to unravel mechanisms that control microbial growth in diverse environments.

143

144 **References and Notes:**

- 145 1. M. Wessling-Resnick, Nramp1 and other transporters involved in metal withholding during
 146 infection. *J Biol Chem* **290**, 18984-18990 (2015).
- 147 2. W. P. Loomis *et al.*, Temporal and anatomical host resistance to chronic *Salmonella* infection is
 148 quantitatively dictated by Nramp1 and influenced by host genetic background. *PLoS One* **9**,
 149 e111763 (2014).
- 150 3. B. Claudi *et al.*, Phenotypic variation of *Salmonella* in host tissues delays eradication by
 151 antimicrobial chemotherapy. *Cell* **158**, 722-733 (2014).
- 152 4. W. H. Benjamin, Jr., P. Hall, S. J. Roberts, D. E. Briles, The primary effect of the *Ity* locus is on
 153 the rate of growth of *Salmonella typhimurium* that are relatively protected from killing. *J*
 154 *Immunol.* **144**, 3143-3151 (1990).
- 155 5. N. A. Burton *et al.*, Disparate impact of oxidative host defenses determines the fate of *Salmonella*
 156 during systemic infection in mice. *Cell Host & Microbe* **15**, 72-83 (2014).
- 157 6. J. K. White, P. Mastroeni, J. F. Popoff, C. A. Evans, J. M. Blackwell, Slc11a1-mediated
 158 resistance to *Salmonella enterica* serovar Typhimurium and *Leishmania donovani* infections does
 159 not require functional inducible nitric oxide synthase or phagocyte oxidase activity. *J Leukoc Biol*
 160 **77**, 311-320 (2005).
- 161 7. M. Nairz *et al.*, Slc11a1 limits intracellular growth of *Salmonella enterica* sv. Typhimurium by
 162 promoting macrophage immune effector functions and impairing bacterial iron acquisition. *Cell*
 163 *Microbiol* **11**, 1365-1381 (2009).
- 164 8. S. Campoy *et al.*, Role of the high-affinity zinc uptake *znuABC* system in *Salmonella enterica*
 165 serovar Typhimurium virulence. *Infect Immun* **70**, 4721-4725 (2002).
- 166 9. P. Petrarca, S. Ammendola, P. Pasquali, A. Battistoni, The Zur-regulated ZinT protein is an
 167 auxiliary component of the high-affinity ZnuABC zinc transporter that facilitates metal
 168 recruitment during severe zinc shortage. *J Bacteriol* **192**, 1553-1564 (2010).
- 169 10. T. D. Lockwood, Lysosomal metal, redox and proton cycles influencing the CysHis cathepsin
 170 reaction. *Metallomics : integrated biometal science* **5**, 110-124 (2013).
- 171 11. K. McGourty *et al.*, *Salmonella* inhibits retrograde trafficking of mannose-6-phosphate receptors
 172 and lysosome function. *Science* **338**, 963-967 (2012).
- 173 12. P. Mastroeni *et al.*, Antimicrobial actions of the NADPH phagocyte oxidase and inducible nitric
 174 oxide synthase in experimental salmonellosis. II. Effects on microbial proliferation and host
 175 survival in vivo. *J Exp Med* **192**, 237-248 (2000).
- 176 13. D. E. Brown *et al.*, Increased ferroportin-1 expression and rapid splenic iron loss occur with
 177 anemia caused by *Salmonella enterica* Serovar Typhimurium infection in mice. *Infect Immun* **83**,
 178 2290-2299 (2015).
- 179 14. D. E. Brown *et al.*, *Salmonella enterica* causes more severe inflammatory disease in C57/BL6
 180 Nramp1G169 mice than Sv129S6 mice. *Vet Pathol* **50**, 867-876 (2013).
- 181 15. R. E. Fleming *et al.*, Mouse strain differences determine severity of iron accumulation in Hfe
 182 knockout model of hereditary hemochromatosis. *Proc Natl Acad Sci U S A* **98**, 2707-2711 (2001).
- 183 16. K. M. Papp-Wallace *et al.*, The CorA Mg²⁺ channel is required for the virulence of *Salmonella*
 184 *enterica* serovar Typhimurium. *J Bacteriol* **190**, 6517-6523 (2008).
- 185 17. S. Chamnongpol, E. A. Groisman, Mg²⁺ homeostasis and avoidance of metal toxicity. *Mol*
 186 *Microbiol* **44**, 561-571 (2002).
- 187 18. A. B. Blanc-Potard, E. A. Groisman, The *Salmonella selC* locus contains a pathogenicity island
 188 mediating intramacrophage survival. *EMBO J.* **16**, 5376-5385 (1997).
- 189 19. M. D. Snavely, S. A. Gravina, T. T. Cheung, C. G. Miller, M. E. Maguire, Magnesium transport
 190 in *Salmonella typhimurium*. Regulation of *mgtA* and *mgtB* expression. *J Biol Chem* **266**, 824-829
 191 (1991).

- 192 20. E. Choi *et al.*, Elongation factor P restricts *Salmonella's* growth by controlling translation of a
193 Mg(2+) transporter gene during infection. *Sci Rep* **7**, 42098 (2017).
- 194 21. J. R. Forbes, P. Gros, Iron, manganese, and cobalt transport by Nramp1 (Slc11a1) and Nramp2
195 (Slc11a2) expressed at the plasma membrane. *Blood* **102**, 1884-1892 (2003).
- 196 22. M. E. Maguire, J. A. Cowan, Magnesium chemistry and biochemistry. *Biometals : an
197 international journal on the role of metal ions in biology, biochemistry, and medicine* **15**, 203-
198 210 (2002).
- 199 23. A. M. Romani, Cellular magnesium homeostasis. *Arch Biochem Biophys* **512**, 1-23 (2011).
- 200 24. N. Martin-Orozco *et al.*, Visualization of vacuolar acidification-induced transcription of genes of
201 pathogens inside macrophages. *Mol Biol Cell* **17**, 498-510 (2006).
- 202 25. C. E. Outten, T. V. O'Halloran, Femtomolar sensitivity of metalloregulatory proteins controlling
203 zinc homeostasis. *Science* **292**, 2488-2492 (2001).
- 204 26. R. L. Smith, M. T. Kaczmarek, L. M. Kucharski, M. E. Maguire, Magnesium transport in
205 *Salmonella typhimurium*: regulation of *mgtA* and *mgtCB* during invasion of epithelial and
206 macrophage cells. *Microbiology* **144**, 1835-1843 (1998).
- 207 27. P. Cuellar-Mata *et al.*, Nramp1 modifies the fusion of *Salmonella typhimurium*-containing
208 vacuoles with cellular endomembranes in macrophages. *J Biol Chem* **277**, 2258-2265 (2002).
- 209 28. J. H. Shin *et al.*, Transport of magnesium by a bacterial Nramp-related gene. *PLoS Genet* **10**,
210 e1004429 (2014).
- 211 29. A. T. Bozzi *et al.*, Conserved methionine dictates substrate preference in Nramp-family divalent
212 metal transporters. *Proc Natl Acad Sci U S A* **113**, 10310-10315 (2016).
- 213 30. M. P. Hensley *et al.*, Characterization of Zn(II)-responsive ribosomal proteins YkgM and L31 in
214 *E. coli*. *J Inorg Biochem* **111**, 164-172 (2012).
- 215 31. S. K. Hoiseth, B. A. Stocker, Aromatic-dependent *Salmonella typhimurium* are non-virulent and
216 effective as live vaccines. *Nature* **291**, 238-239 (1981).
- 217 32. C. Kroger *et al.*, The transcriptional landscape and small RNAs of *Salmonella enterica* serovar
218 Typhimurium. *Proc Natl Acad Sci U S A* **109**, E1277-1286 (2012).
- 219 33. C. Rollenhagen, M. Sorensen, K. Rizos, R. Hurvitz, D. Bumann, Antigen selection based on
220 expression levels during infection facilitates vaccine development for an intracellular pathogen.
221 *Proc Natl Acad Sci U S A* **101**, 8739-8744 (2004).
- 222 34. A. Hecht *et al.*, Measurements of translation initiation from all 64 codons in *E. coli*. *Nucleic
223 Acids Res* **45**, 3615-3626 (2017).
- 224 35. C. Rollenhagen, D. Bumann, *Salmonella enterica* highly expressed genes are disease specific.
225 *Infect.Immun.* **74**, 1649-1660 (2006).
- 226 36. X. J. Yu, K. McGourty, M. Liu, K. E. Unsworth, D. W. Holden, pH sensing by intracellular
227 *Salmonella* induces effector translocation. *Science* **328**, 1040-1043 (2010).
- 228 37. B. Chen *et al.*, Dynamic imaging of genomic loci in living human cells by an optimized
229 CRISPR/Cas system. *Cell* **155**, 1479-1491 (2013).
- 230 38. S. Haueter *et al.*, Genetic vasectomy-overexpression of Prm1-EGFP fusion protein in elongating
231 spermatids causes dominant male sterility in mice. *Genesis (New York, N.Y. : 2000)* **48**, 151-160
232 (2010).
- 233 39. A. J. Grant *et al.*, Modelling within-host spatiotemporal dynamics of invasive bacterial disease.
234 *PLoS Biol* **6**, e74 (2008).
- 235 40. B. Steeb *et al.*, Parallel exploitation of diverse host nutrients enhances salmonella virulence. *PLoS
236 Pathog* **9**, e1003301 (2013).
- 237 41. R. Bruderer, O. M. Bernhardt, T. Gandhi, L. Reiter, High-precision iRT prediction in the targeted
238 analysis of data-independent acquisition and its impact on identification and quantitation.
239 *Proteomics* **16**, 2246-2256 (2016).
- 240 42. L. E. Edgington, M. Bogyo, In vivo imaging and biochemical characterization of protease
241 function using fluorescent activity-based probes. *Current protocols in chemical biology* **5**, 25-44
242 (2013).

- 243 43. M. Miethke, M. A. J. M. M. B. R. Marahiel, Siderophore-based iron acquisition and pathogen
244 control. *J Biol Chem* **287**, 413-451 (2007).
- 245 44. L. R. Devireddy, D. O. Hart, D. H. Goetz, M. R. Green, A mammalian siderophore synthesized
246 by an enzyme with a bacterial homolog involved in enterobactin production. *Cell* **141**, 1006-1017
247 (2010).
- 248 45. C. Correnti, R. K. Strong, Mammalian siderophores, siderophore-binding lipocalins, and the
249 labile iron pool. *J Biol Chem* **287**, 13524-13531 (2012).
- 250 46. A. Ilari, F. Alaleona, P. Petrarca, A. Battistoni, E. Chiancone, The X-ray structure of the zinc
251 transporter ZnuA from *Salmonella enterica* discloses a unique triad of zinc-coordinating
252 histidines. *J Mol Biol* **409**, 630-641 (2011).
- 253 47. M. B. Moncrief, M. E. Maguire, Magnesium and the role of MgtC in growth of *Salmonella*
254 typhimurium. *Infect Immun* **66**, 3802-3809 (1998).
- 255 48. E. Alix, A. B. Blanc-Potard, Peptide-assisted degradation of the *Salmonella* MgtC virulence
256 factor. *Embo j* **27**, 546-557 (2008).
- 257 49. A. Sevostyanova, E. A. Groisman, An RNA motif advances transcription by preventing Rho-
258 dependent termination. *Proc Natl Acad Sci U S A* **112**, E6835-6843 (2015).
- 259 50. G. Zhao, W. Kong, N. Weatherspoon-Griffin, J. Clark-Curtiss, Y. Shi, Mg²⁺ facilitates leader
260 peptide translation to induce riboswitch-mediated transcription termination. *Embo j* **30**, 1485-
261 1496 (2011).
- 262 51. S. V. Spinelli, L. B. Pontel, E. Garcia Vescovi, F. C. Soncini, Regulation of magnesium
263 homeostasis in *Salmonella*: Mg(2+) targets the mgtA transcript for degradation by RNase E.
264 *FEMS Microbiol Lett* **280**, 226-234 (2008).
- 265 52. E. J. Lee, E. A. Groisman, Tandem attenuators control expression of the *Salmonella* mgtCBR
266 virulence operon. *Mol Microbiol* **86**, 212-224 (2012).
- 267 53. E. J. Lee, E. A. Groisman, Control of a *Salmonella* virulence locus by an ATP-sensing leader
268 messenger RNA. *Nature* **486**, 271-275 (2012).
- 269 54. A. R. Gall *et al.*, Mg²⁺ regulates transcription of mgtA in *Salmonella* Typhimurium via
270 translation of proline codons during synthesis of the MgtL peptide. *Proc Natl Acad Sci U S A*
271 **113**, 15096-15101 (2016).
- 272 55. H. Wang *et al.*, Increasing intracellular magnesium levels with the 31-amino acid MgtS protein.
273 *Proc Natl Acad Sci U S A* **114**, 5689-5694 (2017).
- 274 56. E. Choi, K. Y. Lee, D. Shin, The MgtR regulatory peptide negatively controls expression of the
275 MgtA Mg²⁺ transporter in *Salmonella enterica* serovar Typhimurium. *Biochem Biophys Res*
276 *Commun* **417**, 318-323 (2012).
- 277 57. E. Choi *et al.*, Elongation factor P controls translation of the mgtA gene encoding a Mg(2+)
278 transporter during *Salmonella* infection. *MicrobiologyOpen*, e00680 (2018).
- 279 58. A. J. Westermann *et al.*, The Major RNA-Binding Protein ProQ Impacts Virulence Gene
280 Expression in *Salmonella enterica* Serovar Typhimurium. *MBio* **10**, (2019).
- 281 59. N. Jabado *et al.*, Natural resistance to intracellular infections: natural resistance-associated
282 macrophage protein 1 (Nramp1) functions as a pH-dependent manganese transporter at the
283 phagosomal membrane. *J Exp Med* **192**, 1237-1248 (2000).
- 284 60. S. Buracco *et al.*, Dictyostelium Nramp1, which is structurally and functionally similar to
285 mammalian DMT1 transporter, mediates phagosomal iron efflux. *J Cell Sci* **128**, 3304-3316
286 (2015).
- 287 61. M. L. Zaharik *et al.*, The *Salmonella enterica* serovar typhimurium divalent cation transport
288 systems MntH and SitABCD are essential for virulence in an Nramp1G169 murine typhoid
289 model. *Infect Immun* **72**, 5522-5525 (2004).
- 290 62. G. Fritsche, M. Nairz, S. J. Libby, F. C. Fang, G. Weiss, Slc11a1 (Nramp1) impairs growth of
291 *Salmonella enterica* serovar typhimurium in macrophages via stimulation of lipocalin-2
292 expression. *J Leukoc Biol* **92**, 353-359 (2012).

- 293 63. D. J. Hackam *et al.*, Host resistance to intracellular infection: mutation of natural resistance-
 294 associated macrophage protein 1 (Nramp1) impairs phagosomal acidification. *J Exp Med* **188**,
 295 351-364 (1998).
- 296 64. Y. Valdez *et al.*, Nramp1 drives an accelerated inflammatory response during Salmonella-induced
 297 colitis in mice. *Cell Microbiol* **11**, 351-362 (2009).
- 298 65. J. F. Hedges, E. Kimmel, D. T. Snyder, M. Jerome, M. A. Jutila, Solute carrier 11A1 is expressed
 299 by innate lymphocytes and augments their activation. *J Immunol* **190**, 4263-4273 (2013).
- 300 66. G. Fritsche *et al.*, Nramp1 functionality increases inducible nitric oxide synthase transcription via
 301 stimulation of IFN regulatory factor 1 expression. *J Immunol* **171**, 1994-1998 (2003).
- 302 67. C. H. Barton, S. H. Whitehead, J. M. Blackwell, Nramp transfection transfers Ity/Lsh/Bcg-related
 303 pleiotropic effects on macrophage activation: influence on oxidative burst and nitric oxide
 304 pathways. *Molecular medicine (Cambridge, Mass.)* **1**, 267-279 (1995).
- 305 68. S. Soe-Lin *et al.*, Nramp1 promotes efficient macrophage recycling of iron following
 306 erythrophagocytosis in vivo. *Proc Natl Acad Sci U S A* **106**, 5960-5965 (2009).
- 307 69. S. Soe-Lin *et al.*, Both Nramp1 and DMT1 are necessary for efficient macrophage iron recycling.
 308 *Exp Hematol* **38**, 609-617 (2010).
- 309 70. G. Fritsche *et al.*, Modulation of macrophage iron transport by Nramp1 (Slc11a1).
 310 *Immunobiology* **212**, 751-757 (2007).
- 311 71. M. Nairz, I. Theurl, F. K. Swirski, G. Weiss, "Pumping iron"-how macrophages handle iron at the
 312 systemic, microenvironmental, and cellular levels. *Pflugers Arch* **469**, 397-418 (2017).
- 313 72. S. Ammendola *et al.*, High-affinity Zn²⁺ uptake system ZnuABC is required for bacterial zinc
 314 homeostasis in intracellular environments and contributes to the virulence of Salmonella enterica.
 315 *Infect Immun* **75**, 5867-5876 (2007).
- 316 73. S. M. Newton, J. D. Igo, D. C. Scott, P. E. Klebba, Effect of loop deletions on the binding and
 317 transport of ferric enterobactin by FepA. *Mol Microbiol* **32**, 1153-1165 (1999).
- 318 74. K. J. F. m. l. Hantke, Ferrous iron transport mutants in Escherichia coli K12. **44**, 53-57 (1987).
- 319 75. D. G. Kehres, A. Janakiraman, J. M. Schlauch, M. E. Maguire, SitABCD is the alkaline Mn(2+)
 320 transporter of Salmonella enterica serovar Typhimurium. *J Bacteriol* **184**, 3159-3166 (2002).
- 321 76. D. G. Kehres, M. L. Zaharik, B. B. Finlay, M. E. Maguire, The NRAMP proteins of Salmonella
 322 typhimurium and Escherichia coli are selective manganese transporters involved in the response
 323 to reactive oxygen. *Mol Microbiol* **36**, 1085-1100 (2000).
- 324 77. A. Ilari *et al.*, The Salmonella enterica ZinT structure, zinc affinity and interaction with the high-
 325 affinity uptake protein ZnuA provide insight into the management of periplasmic zinc. *Biochim*
 326 *Biophys Acta* **1840**, 535-544 (2014).
- 327 78. N. Taudte, G. Grass, Point mutations change specificity and kinetics of metal uptake by ZupT
 328 from Escherichia coli. *Biometals : an international journal on the role of metal ions in biology,*
 329 *biochemistry, and medicine* **23**, 643-656 (2010).
- 330 79. C. Bradbeer, P. R. Reynolds, G. M. Bauler, M. T. Fernandez, A requirement for calcium in the
 331 transport of cobalamin across the outer membrane of Escherichia coli. *J Biol Chem* **261**, 2520-
 332 2523 (1986).
- 333 80. S. P. Hmiel, M. D. Snavely, C. G. Miller, M. E. Maguire, Magnesium transport in Salmonella
 334 typhimurium: characterization of magnesium influx and cloning of a transport gene. *J Bacteriol*
 335 **168**, 1444-1450 (1986).
- 336 81. M. D. Snavely, J. B. Florer, C. G. Miller, M. E. Maguire, Magnesium transport in Salmonella
 337 typhimurium: 28Mg²⁺ transport by the CorA, MgtA, and MgtB systems. *J Bacteriol* **171**, 4761-
 338 4766 (1989).

339

340 **Acknowledgements:** We would like to thank Beatrice Claudi, Patricia Marini, Pauline Maturana, and
341 Francesca Romana Cianfanelli for help with cloning and animal experiments, Pawel Pelczar (University
342 of Basel Center for Transgenic Models) for help with genome editing, Stella Stevanova and Janine Bögli
343 (Biozentrum FACS Core Facility) for sorting, Sandra Söderholm and Thomas Bock (Biozentrum
344 Proteomics Core Facility) for help with proteomics, and Sarah Thomforde for correcting grammatical
345 errors; **Funding:** This research was supported by Swiss National Science Foundation (Projects
346 310030_156818, 310030_182315) to DB; **Author contributions:** Outlined the study: DB, OC,
347 Performed experiments: OC, Analysed data: OC, DB, Interpreted data: OC, DB, Wrote manuscript: DB
348 with major contribution of OC; **Competing interests:** Authors declare no competing interests; **Data and**
349 **materials availability:** All data is available in the manuscript or the supplementary materials.
350 Correspondence and requests for materials should be addressed to D.B.

351

352 **Supplementary Materials:**

353 Materials and Methods

354 Supplementary Text

355 Figs. S1-S7

356 Table S1

357 References (31-81)

358

359 **Figure legends**

360

361 **Fig. 1. SLC11A1 restricts *Salmonella* proliferation in mouse spleen**

362 (A) Growth of *Salmonella* in spleen of *SLC11A1*^{s/s} (s/s), *SLC11A1*^{r/s} (r/s), *SLC11A1*^{r/r} (r/r), or
363 129S2/SvPasCrl mice (129, data already reported in (3)). Each symbol represents a single
364 mouse (****, $P < 0.0001$; One-way ANOVA of log-transformed values with Holm-Šídák
365 correction for multiple comparisons).

366 (B) *Salmonella in-vivo* division rates as calculated from TIMER^{bac} data shown in Fig. S2D.

367 (C) Median *Salmonella* division rates in different mouse genotypes. Each symbol represents a
368 single mouse (****, $P < 0.0001$; One-way ANOVA of log-transformed values with Holm-
369 Šídák correction).

370 (D) Comparison of *Salmonella* net growth based on spleen loads shown in A, and *Salmonella*
371 division rates (div. rates) shown in C.

372

373 **Fig. 2. SLC11A1 triggers *Salmonella* metal uptake**

374 (A) Proteome comparisons of *Salmonella* sorted from spleen with different *SLC11A1* genotypes
375 ((s/s), day 4 after low-dose infection; (r/s), day 6 after low-dose infection; (r/r), day 4 after
376 high-dose infection). Each circle represents a protein. Metal uptake systems are shown in
377 color. RpmE2 increases cytosolic zinc concentration by replacing the zinc-binding ribosomal
378 subunit L31 (30). Ribosomal proteins (RBS) are shown in black.

379 (B) Fluorescence of *Salmonella* reporter strains for reactive oxygen species (ROS) or reactive
380 nitrogen species (RNS) (grey areas, autofluorescence; dashed lines, threshold for defining
381 “GFP^{hi}” subsets; MFI, median GFP fluorescence intensities of “GFP^{hi}” subsets). Each circle
382 represents a single mouse. More *Salmonella* cells had high P_{hmpA} -activity at day 6 in (r/s)

383 mice consistent with increasing nitric oxide exposure during disease progression (5) (*P*-value
384 for One-way ANOVA with test for linear trend). *In-vitro* induction data for both promoters
385 are shown in Fig. S3b.

386

387 **Fig. 3. SLC11A1 causes a specific requirement for the *Salmonella* magnesium transporter MgtB**

388 (A) Apparent affinities of *Salmonella* uptake systems for various metals (see table S1 for
389 numerical values; TBDT, TonB-dependent transporters). The CbiMNQ system transports
390 Co^{2+} but affinities have not yet been reported.

391 (B) Fitness of *Salmonella* mutants relative to wild-type in mice with different *SLC11A1*
392 genotypes. Data points in the orange area indicate stronger fitness defects in presence of
393 SLC11A1 (dashed ellipses). Averages and standard deviations for three to five mice are
394 shown (****, $P < 0.0001$; **, $P < 0.01$; *, $P < 0.05$; two-tailed t-test with Holm-Šídák
395 correction). Mutant “1” is shown in grey to indicate that some data points were below the
396 limit of detection. Corresponding competitive indices (CI) are shown in Fig. S4.

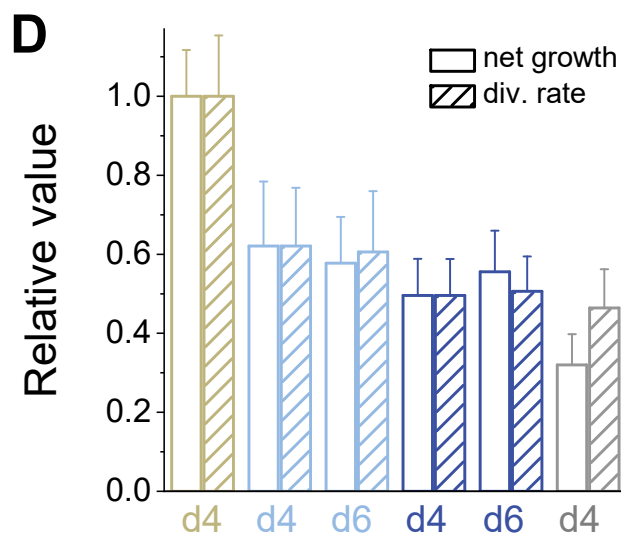
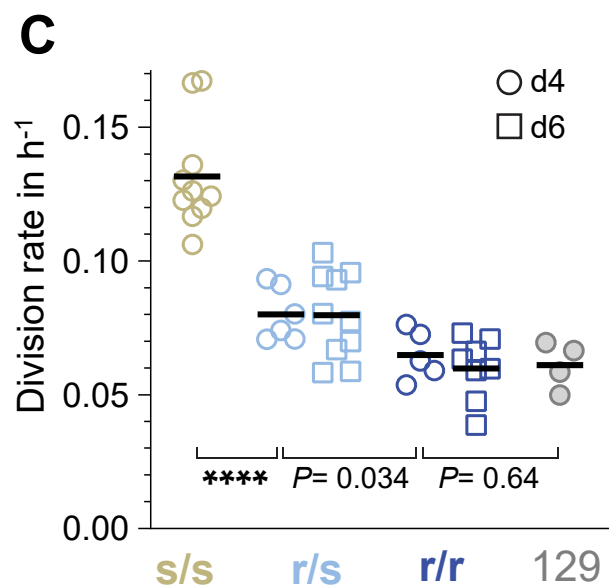
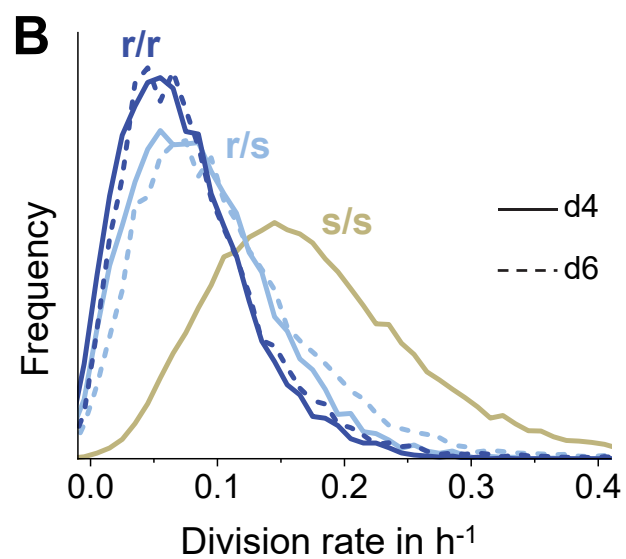
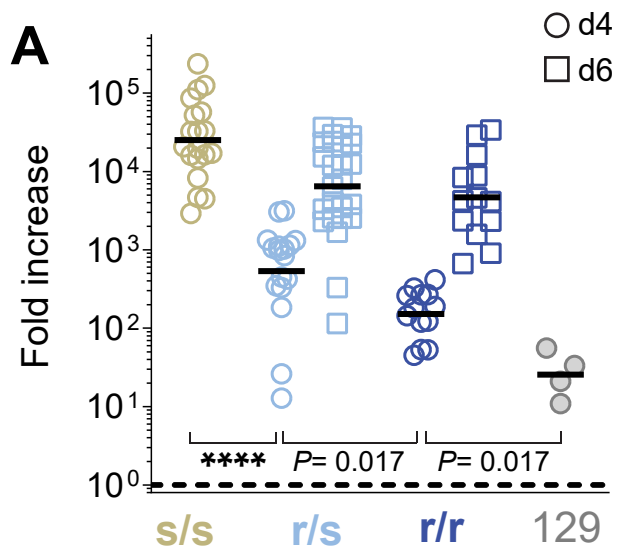
397 (C) Fitness of selected *Salmonella* mutants. Each symbol represents an individual mouse.
398 Statistical significance of differences to fitness in (*s/s*) mice was tested using One-way
399 ANOVA with Holm-Šídák correction (****, $P < 0.0001$; ***, $P < 0.001$; **, $P < 0.01$; *, $P <$
400 0.05).

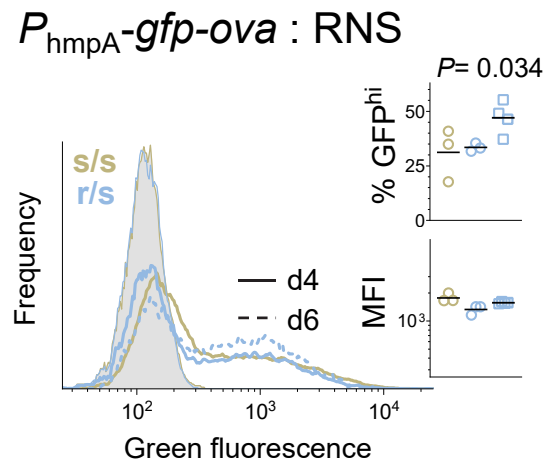
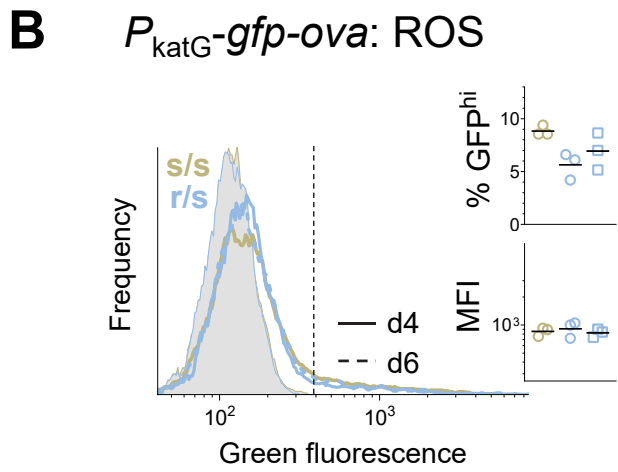
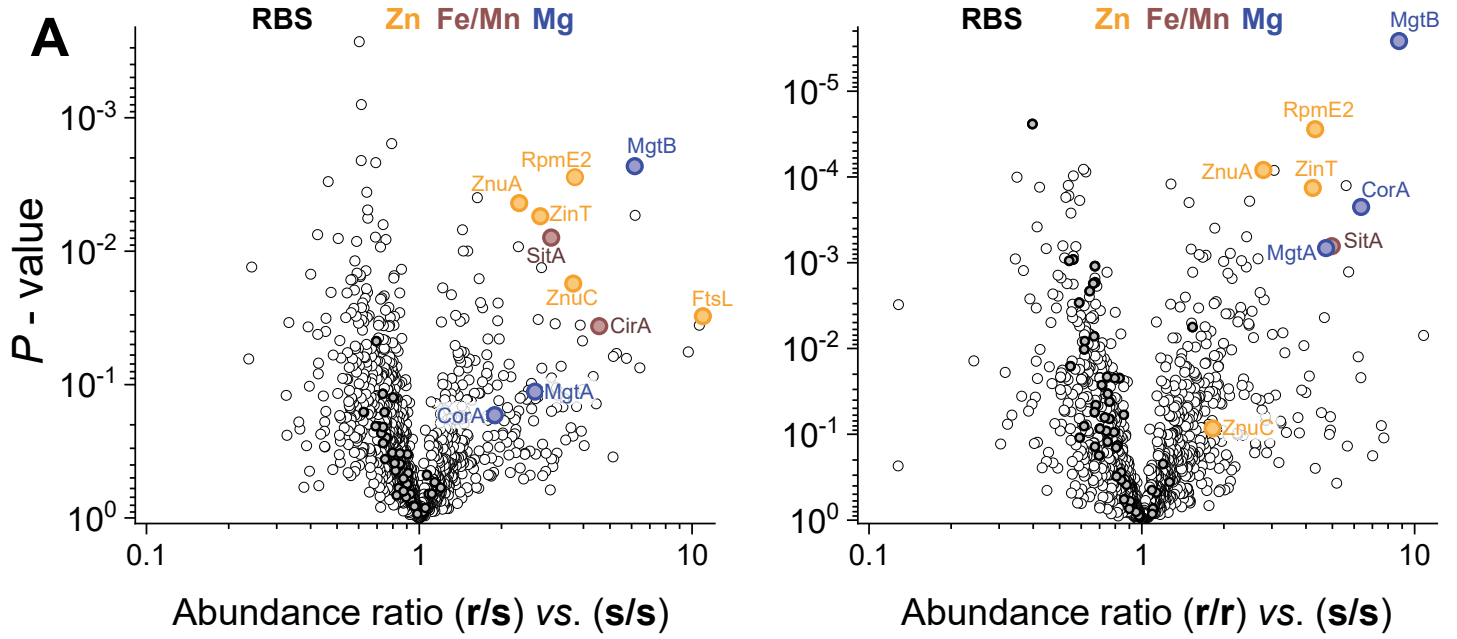
401 (D) Growth of wild-type and mutant *Salmonella in vitro*. Minimal medium was supplemented
402 with 1 μM FeSO_4 and variable amounts of 2,2'-bipyridyl (left side), or variable amounts of
403 FeSO_4 and no 2,2'-bipyridyl (right side). Individual data and means of two biological
404 replicates are shown. Coarse estimates for *in-vivo* iron availabilities are shown below.

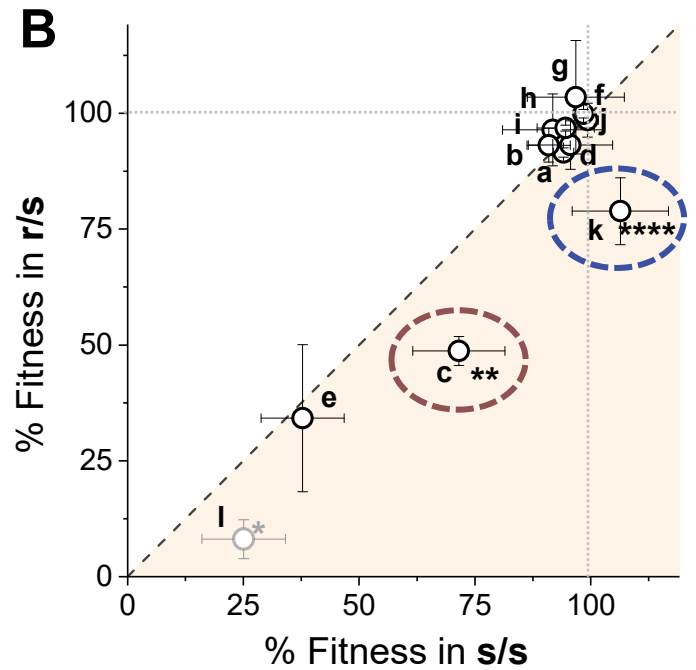
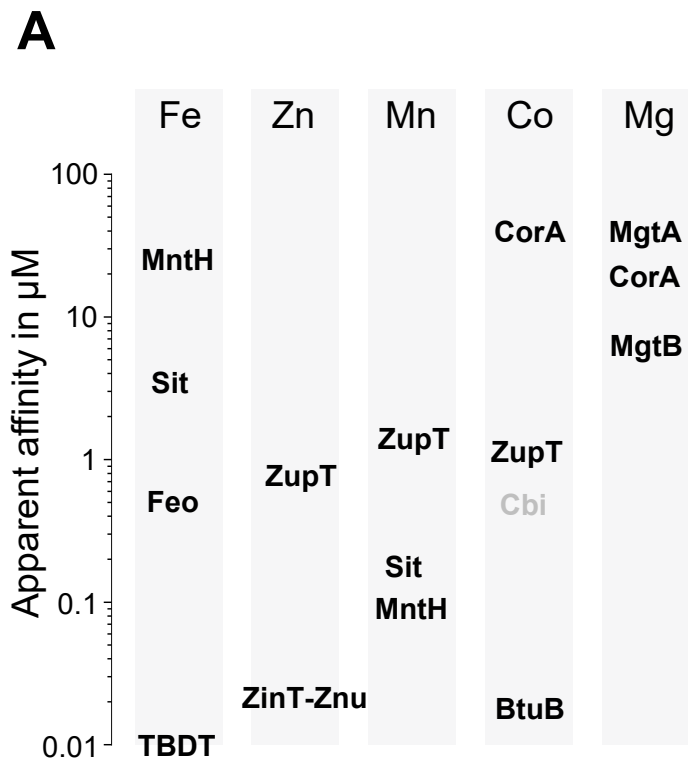
405

406 **Fig. 4. Equivalent *Salmonella* single-cell responses to SLC11A1 and magnesium deprivation**

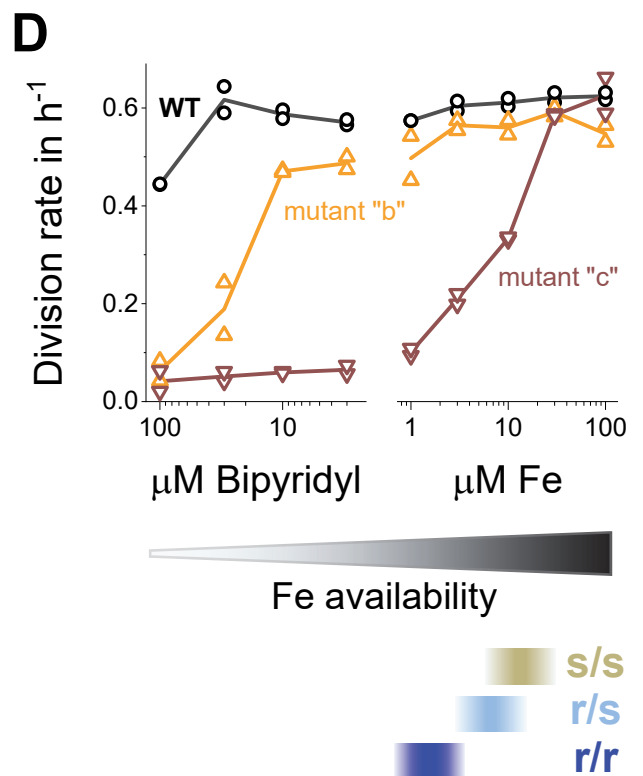
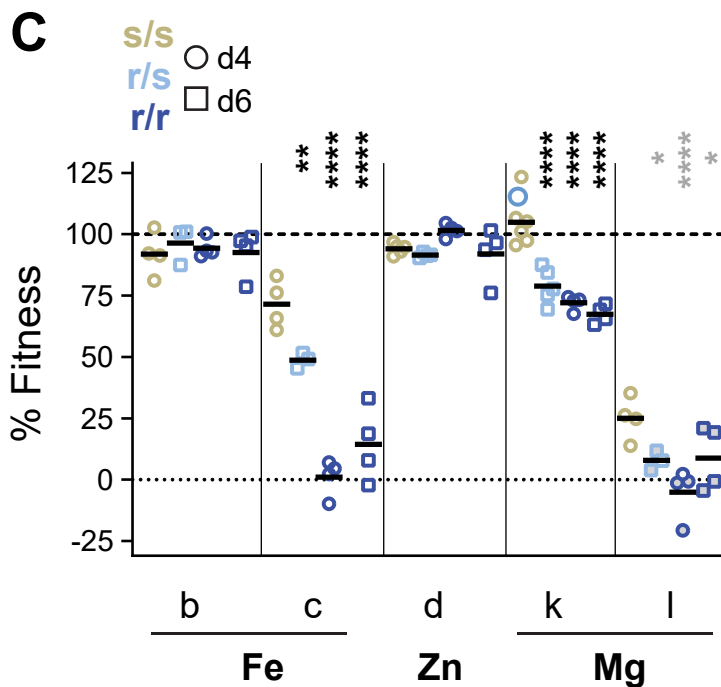
- 407 **(A)** Single-cell density maps of *Salmonella* division rates and $P_{\text{mgtCBRcigR}}$ activities (pooled data
408 from three to four mice). The dashed lines represent a fit to median values of (r/s) and (r/r)
409 mice as shown in C. The dotted vertical lines show the median *Salmonella* division rate in
410 (s/s) mice.
- 411 **(B)** Squared values (“goodness-of-fit”) for Spearman’s rank correlation between single-cell
412 *Salmonella* division rates and promoter activities. Each symbol represents a single mouse
413 (****, $P < 0.0001$; ***, $P < 0.001$; statistical significance of difference to values for (s/s) mice
414 based on One-way ANOVA with Holm-Šídák correction).
- 415 **(C)** Median division rates and $P_{\text{mgtCBRcigR}}$ activities for *Salmonella* and mouse genotypes shown in
416 A. Each symbol represents a single mouse. The black line represents a linear regression of
417 data for (r/s) and (r/r) mice with 90% confidence bands shown as dashed lines. The inset is an
418 enlargement of the most informative data range.

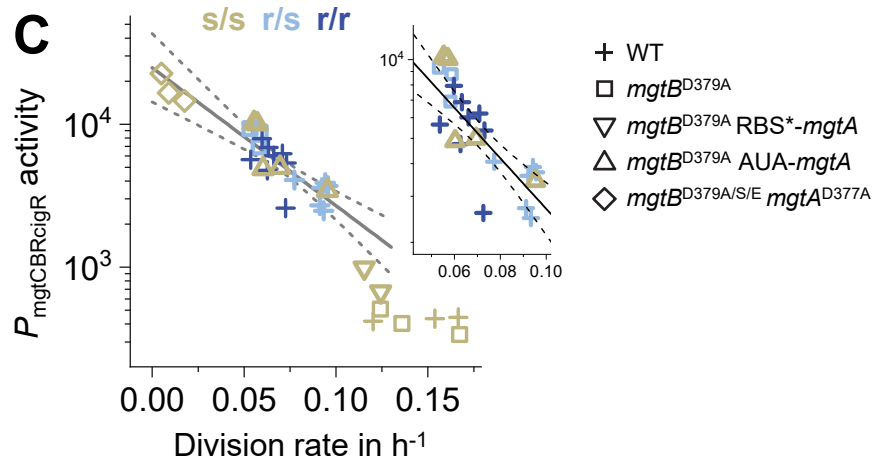
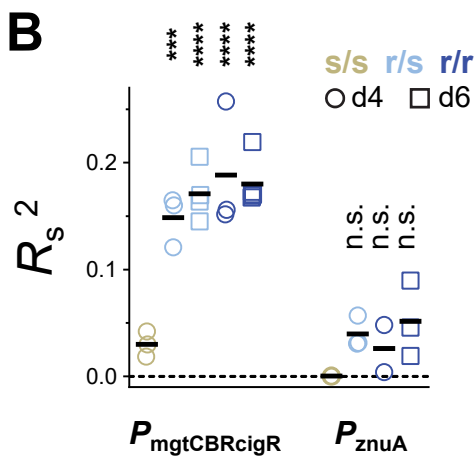
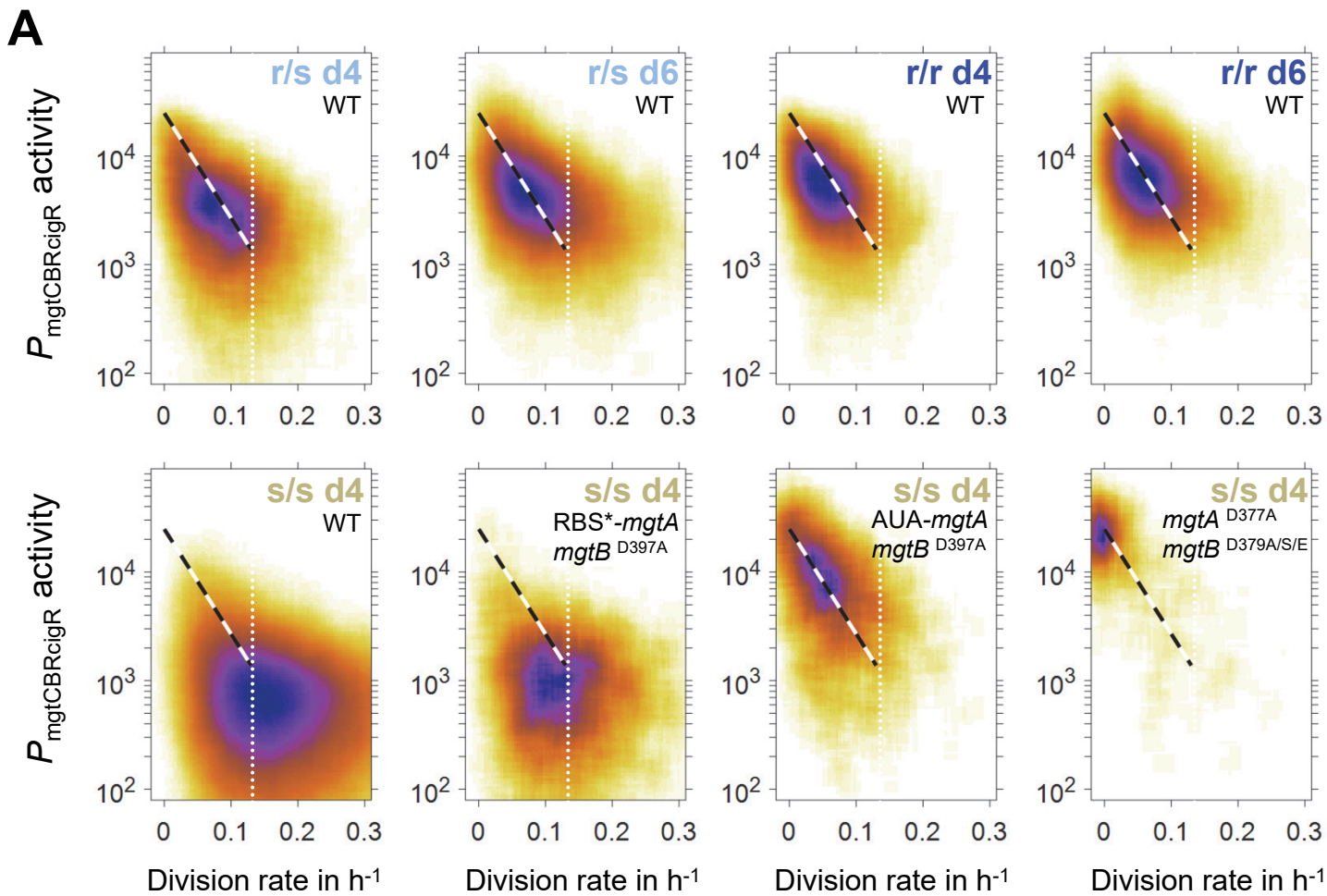






- | | | | |
|-----------|--|-----------|---|
| Fe | a: <i>feoABC entC</i> | Mn | f: <i>mntH sitABCD zupT</i> |
| | b: <i>feoABC tonB</i> | | g: <i>mntP</i> |
| | c: <i>feoABC entC sitABCD mntH</i> | Cu | h: <i>copA golT cueO cueP</i> |
| Zn | d: <i>zinT znuA^{Δ138-160}</i> | Co | i: <i>tonB cbiMNQO</i> |
| | e: <i>znuABC</i> | | j: <i>mgtA^{D377A}</i> |
| | | Mg | k: <i>mgtB^{D379A}</i> |
| | | | l: <i>mgtA^{D377A} mgtB^{D379A}</i> |







Supplementary Materials for

Host-resistance factor SLC11A1 restricts *Salmonella* growth through magnesium deprivation

Olivier Cunrath, Dirk Bumann

Correspondence to: dirk.bumann@unibas.ch

This PDF file includes:

Materials and Methods
Supplementary Text
Figs. S1 to S7
Table S1

Materials and Methods

Microbiology and Molecular Biology

Salmonella strains used in this study were based on SME51, a prototrophic *hisG*^{Leu69} derivative of *Salmonella enterica* serovar Typhimurium SL1344 (1-3). *Salmonella* strains with gene deletions (preserving a nonapeptide with the first five and last four amino acids, and the stop codon) or point mutations were obtained by two consecutive single-crossovers with positive selection for resistance to kanamycin and negative selection against levan sucrose-mediated sensitivity to sucrose. MgtA abundance was modulated by replacing the wild-type ribosomal binding site gcgGagggga with the variant gcgCagggga (4), or by replacing the wild-type initiation codon AUG with AUA (5).

Reporter strains carried low-copy episomal pSC101-derivatives with constitutive expression of *mCherry* or *mtagbfp2* from the *P_{ybaJ}* promoter (6), and fusions of candidate promoters (*P_{mgtCBRcigR}*, chromosomal coordinates in SL1344 GenBank: FQ312003.1: 3,987,050 - 3,986,453; *P_{znuA}*, 1,945,050 - 1,944,940) driving expression of *mtagbfp2-ova* coding for a degradable variant (4) of the blue fluorescent protein mTagBFP2. Bacteria were grown in Lennox lysogeny broth (LB) or in MES-MM (100 mM MES-KOH pH 5.5, 0.4% glycerol, 15 mM NH₄Cl, 1.5 mM K₂SO₄, 3 mM KH₂PO₄) (7) containing 5 mM ascorbate.

Generation of C57BL/6-SLC11A1^{r/s} mice

SLC11A1 was edited using Cas9/CRISP directly in fertilized mouse oocytes. C57BL/6J female mice underwent ovulation induction by i.p. injection of 5 IU equine chorionic gonadotrophin (PMSG; Folligon–InterVet), followed by i.p. injection of 5 IU human chorionic gonadotropin (Pregnyl–Essex Chemie) 48 h later. For zygote recovery, C57BL/6J females were mated with males of the same strain immediately after the administration of human chorionic gonadotropin. All zygotes were collected from oviducts 24 h after the human chorionic gonadotropin injection, and were then freed from any remaining cumulus cells by a 1–2 min treatment of 0.1% hyaluronidase (Sigma-Aldrich) dissolved in M2 medium (Sigma-Aldrich). Mouse embryos were cultured in M16 (Sigma-Aldrich) medium at 37°C and 5% CO₂. For micromanipulation, embryos were transferred into M2 medium. All microinjections were performed using a microinjection system comprised of an inverted microscope equipped with Nomarski optics (Nikon), a set of micromanipulators (Narashige), and a FemtoJet microinjection unit (Eppendorf). Injection solution containing 300 ng/ul in-vitro transcribed sgRNA^(F+E) (8) targeting the sequence atcagtacaccgtccacagcgg, 20 ng/ul ssDNA homologous recombination template

aagctggccaagctatttgggttctgactccccagatctgggtcaggctgattgatctgcctacttggttcagcatcccattatggggagg agttctgatcaccattgtggacaccttcttctcttcttcttggataactatggtgagagaaagcccctcatcttgggggataa (IDT), 50 ng/μl Cas9 protein (Toolgenl) was microinjected into the male pronuclei of fertilized mouse oocytes until 20-30% distension of the organelle was observed. Embryos that survived the microinjection were transferred on the same day into the oviducts of 8 to 16 week-old pseudo-pregnant Cr1:CD1(ICR) females (0.5 days after coitus) that had been mated with sterile genetically vasectomized males (9) half a day before. Pregnant females were allowed to deliver and raise their pups until weaning age.

A female mouse carrying one *SLC11A1*^r (G169) allele was identified using PCR of genomic DNA with primers oOPC-048, aagctatttgggttctgactccc; oOPC-049, cccaagatgaggggcttctctc, and oOPC-525, tggatcagaactcctcccataat. This yielded two fragments of 160 and 80 bp, instead of only a single fragment with 160 bp for parental C57BL/6J mice

carrying two *SLC11A1*^s (D169) alleles. We used <http://crispr.mit.edu/> to predict 49 potential off-target sites with scores between 0.2 and 1.4 (the on-target score was 82). We sequenced the top two off-targets (chr6:146,977,326; chr11:96,132,107) and two other off-targets, that were the only ones associated with exons (chr9:96,844,606, USCS gene NM_145134; chr9:106,366,025, NM_021567) and found no mutation in any of them compared to the parental C57BL/6J genome. The founder mouse was mated with C57BL/6J males. Offspring was characterized with the same PCR and an additional PCR with primers oOPC-048, oOPC-49, and oOPC-524, atcagtacaccgtcccacagc, which yielded two fragments (80, 160 bp) if at least one *SLC11A1*^s allele was present, but only a single 160 bp fragment for *SLC11A1*^{r/r} homozygotes.

Mouse Infections and Tissue Collection

All animal experiments were approved (license 2239, Kantonales Veterinäramt Basel) and performed according to local guidelines (Tierschutz-Verordnung, Basel) and the Swiss animal protection law (Tierschutz-Gesetz). Sex-matched 10 to 16 week-old littermates were infected by tail vein injection of *Salmonella* strain mixtures containing 1,000 to 3,000 CFU in 100 μ l PBS. The inoculum size was determined by plating for each infection. Mice were scored daily for disease signs (unprovoked behavior: normal – 0, minor changes – 1, less mobile and isolated – 2, restless and very still – 3; provoked behavior: responsive & alert – 0, unresponsive & not alert – 3; physical appearance: normal – 0, lack of grooming – 1, piloerection – 2, hunched up – 3, eyes half closed – 4; clinical signs: normal respiratory rate – 0, slight changes – 1, decreased rate and abdominal breathing – 2, marked abdominal breathing & cyanosis – 3; hydration status: normal – 0, dehydrated – 5; strength (grip test): grab and pull – 0, grab but no pull – 2, neither grab nor pull – 4; surface temperature was not indicative of progressive disease; maximum combined score 22). Four or six days post-infection, the mice were euthanized with carbon dioxide and the spleen was prepared. *Salmonella* load was determined by plating and flow cytometry. Fitness was calculated as $\log_2(\text{FI})$ with FI corresponding to the fold increase starting from the initial spleen colonization (around 20% of the total inoculum (10)) to the final spleen load. The relative fitness value of co-administered wild-type *Salmonella* was set to 100%. We also determined the more commonly used read-out “competitive index” (CI) by dividing the output ratio (mutant/wild-type) by the inoculum ratio (mutant/wild-type) (fig. S3). In contrast to relative fitness, CI values increasingly diverge from 1 with ongoing division. Because *Salmonella* divided more slowly in (r/s) mice compared to (s/s) mice (Fig. 1F), CI values underestimated the mutant phenotypes in (r/s) mice.

We estimated sample size by a sequential statistical design. We first infected two to three mice based on effect sizes and variation observed in our previous studies (11), and used the results to estimate group sizes for obtaining statistical significance with sufficient power. The experiments were neither randomized nor blind. However, flow cytometry analysis was carried out using an automated unbiased approach (see Flow Cytometry section).

Flow Cytometry

The spleen was homogenized in ice-cold PBS containing 0.2% Triton X-100. All samples were kept on ice until and during analysis. Large host cell fragments were removed by centrifugation at 500xg for 5 min. Relevant spectral parameters were recorded in a FACS Fortessa II equipped with 405 nm, 488 nm and 561 nm lasers (Becton Dickinson), using thresholds on SSC and FSC to exclude electronic noise. We used the following channels: mTagBFP2, excitation 405 nm, emission 460-480 nm (“blue”); GFP and green TIMER^{bac}

component, excitation 488 nm, emission 502-525 nm (“green”); mCherry and orange TIMER^{bac} component, excitation 561 nm, emission 595-664 nm (“orange”); yellow autofluorescence channels, excitation 445 nm, emission 573-613 nm; or excitation 488 nm, emission channels 533-551 nm, 573-613 nm. NeonGreen- or YPet-expressing *Salmonella* cells were purified from infected spleen homogenates using an Aria IIIu cell sorter (BD Biosciences) using excitation 488 nm and emission channels 499-529 nm (NeonGreen) or 533-551 nm (YPet), and 573-613 nm for separating host autofluorescence, in presence of 170 mg l⁻¹ chloramphenicol to prevent *de-novo* protein biosynthesis. Data were processed with FlowJo, FCS Express, and MATLAB. TIMER^{bac} fluorescence log color ratios (green/orange) were converted into *Salmonella* division rates based on a previously established calibration data (3).

Proteomics

We purified between 1.5x10⁶ and 9x10⁶ *Salmonella* cells from individual *SLC11A1*^{s/s}, *SLC11A1*^{v/s}, and *SLC11A1*^{v/r} mice (three to five mice for each genotype). We processed the sorted *Salmonella* with the sample preparation kit from PreOmics (PreOmics GmbH, Germany) according to the protocol version 2.2. The cell pellets were lysed in 50 µl of the provided lysis buffer and heated for 5 min at 95 °C. The samples were sonicated with a Bioruptor (Diagenode) applying the standard 10 min method (30 s on, 30 s off, 10 cycles). The protein concentration was determined by BCA assay (Thermo-Pierce, 23252). A maximum of 100 µg protein was digested at 37 °C for four hours. After clean-up according to the manufacturer’s procedure, the peptides were dried in a vacuum centrifuge and frozen at -20 °C until further use.

Data independent analysis (DIA) was performed using a previously described method on an high mass-accuracy and resolution mass spectrometer (Q-Exactive HF coupled online to an Easy-nLC-system, Thermo-Fisher Scientific) (12). Peptide separation was performed over 90 min. The HRM DIA method contained an MS1 scan (target setting: 5e6 ions, maximum accumulation time: 100 ms, scan range: 400-1220 m/z, resolution: 120,000 FWHM) followed by acquisition of 38 DIA windows (target setting: 3e6 ions, maximum accumulation time: auto, resolution: 30,000 FWHM). Stepped collision energy was 22.5%, 25%, 27.5%. Spectra were recorded in profile mode.

Data files were converted to htrms file format using HTRMS Converter (Biognosys AG, Switzerland). Converted files were imported into Spectronaut 11 (Biognosys AG, Switzerland) using default parameters with the following exceptions for the quantification settings: no cross run normalization and only proteotypic peptides allowed. An in-house *Salmonella enterica* serovar Typhimurium SL1344 spectral library was generated from *Salmonella* grown under different standard media conditions and from *Salmonella* purified from mouse spleen. This spectral library was merged with an in-house generated mouse spectral library. Peptide and protein identification was performed allowing a q-value of 0.01. Protein abundance was calculated from the protein group quantity calculation (PG quantity) as provided by the software and the protein’s molecular weight. Samples were normalized assuming a total protein content of 190 fg per cell.

Confocal microscopy

2-3mm thick spleen sections from mice that received 20 nanomole of the cathepsin probe iABP (13) (Vergent Bioscience) i.v. 1 h before harvesting, were fixed with fresh 4% paraformaldehyde at 4°C for 4 h, followed by incubating in increasing sucrose concentrations from 10%-40% at 4°C. After overnight incubation in 40% sucrose, tissue was rapidly frozen in

embedding media (Tissue-Tek® O.C.T; Sakura), and then stored at -80°C. 10-14 µm thick cryosections were cut, put on coated glass cover slips (Thermo Scientific), and dried in a desiccator. Sections were mounted in fluorescence mounting medium (Dako), and examined with a Zeiss LSM 880 confocal microscope using glycerol 40X and 63X objectives.

Statistics

Statistical tests were performed with GraphPad Prism 8.0.2 as indicated in the figure legends.

Supplementary Text

Genotypes of *Salmonella* iron-transport mutants:

“a”, *feoABC entC*: lacks the high-affinity transporter for Fe²⁺ FeoABC and is defective for biosynthesis of *Salmonella* siderophores for high-affinity uptake of Fe³⁺

“b”, *feoABC tonB*: lacks the high-affinity transporter for Fe²⁺ FeoABC and TonB required for high-affinity uptake of ferri-siderophores (14), including mammalian ferri-2,5-dihydroxybenzoic acid (gentisic acid) (15, 16)

“c”, *feoABC entC sitABCD mntH*: lacks the high-affinity transporter for Fe²⁺ FeoABC and moderate-affinity transporters for Fe²⁺ SitABCD and MntH (which also transport Mn²⁺ with high affinity), and is defective for biosynthesis of *Salmonella* siderophores for high-affinity uptake of Fe³⁺

Zinc-transport mutants

“d”, *zinT znuA*^{Δ138-160}: lacks the auxiliary periplasmic Zn²⁺-binding protein ZinT and carries a variant of the other periplasmic Zn²⁺-binding protein ZnuA with reduced loading capacity for Zn²⁺ to the membrane transporter ZnuBC (17, 18)

“e”, *znuABC*: lacks the high-affinity Zn²⁺ ABC transporter

Manganese-transport mutants

“f” *mntH sitABCD zupT*: lacks the high-affinity transporters for Mn²⁺ SitABCD and MntH (which also transport Fe²⁺ with moderate affinity) and the moderate-affinity Mn²⁺ transporter ZupT (which also transports Zn²⁺ and Co²⁺)

g: *mntP*: lacks the Mn²⁺ exporter MntP

Copper-transport mutant

h: *copA golT cueO cueP*: lacks export and binding proteins involved in copper resistance

Cobalt-transport mutant

i: *tonB cbiMNQO*: lacks TonB-dependent vitamin B12 uptake through BtuB and the high-affinity Co²⁺ transporter CbiMNQO

Magnesium-transport mutants

We inactivated MgtA and MgtB by mutating crucial aspartate residues (19) instead of full gene deletions, in order to minimize polar effects on their complex genetic loci with multiple promoters, protein coding genes, non-coding RNAs, and extensive post-transcriptional regulation (19-30).

j: *mgtA*^{D377A}: inactive moderate-affinity transporter MgtA

k: *mgtB*^{D379A}: inactive moderate-affinity transporter MgtB

l: *mgtA*^{D377A} *mgtB*^{D379A}: both MgtA and MgtB are inactive

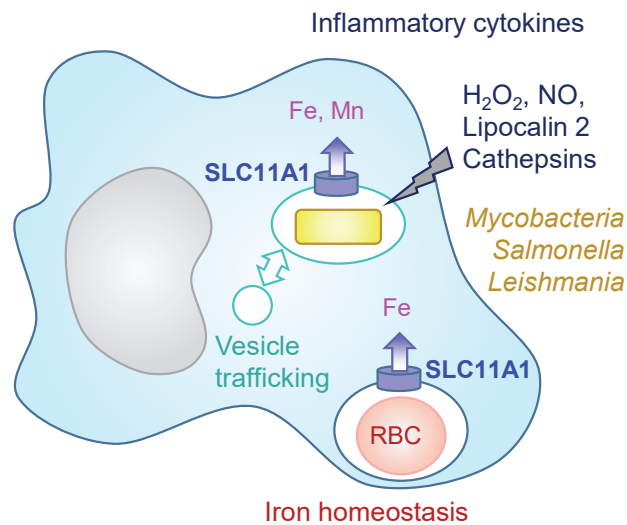


Fig. S1. Pleiotropic impact of SLC11A1 during infection with diverse intracellular pathogens. SLC11A1 transports the divalent metal cations Fe²⁺, Mn²⁺ out of phagosomes (31-33), and may deprive vacuolar pathogens of these essential micronutrients (34-36). In addition, SLC11A1 has pleiotropic effects on phagosome maturation (37-39); pro-inflammatory cytokines (34, 40) and activation of innate lymphocytes (41); generation of antimicrobial effector molecules such as nitric oxide (NO) (34, 42, 43), reactive oxygen species such as hydrogen peroxide (H₂O₂) (36, 43), and lipocalin 2 (36). SLC11A1 contributes to iron homeostasis by facilitating recycling of iron from aged red blood cells (RBC) (44-47).

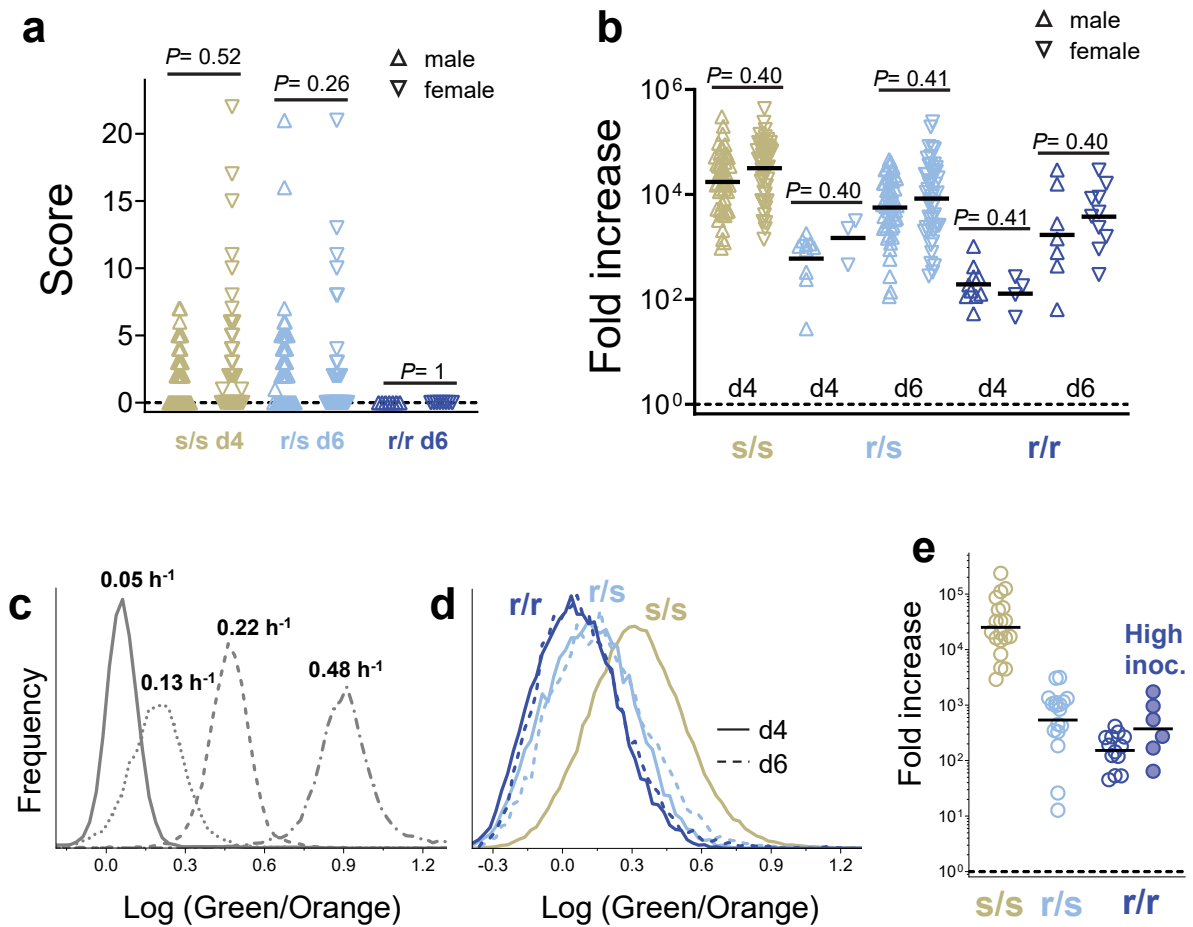


Fig. S2. Responses to *Salmonella* infection in different mouse genotypes. (a) Scores for disease symptoms in male and female mice (d4, day 4; d6, day 6). Each symbol represents a single mouse. Statistical differences between sexes were tested according to Kolmogorov-Smirnov with Holm-Šídák correction for multiple testing. (b) Growth of *Salmonella* in spleen of male and female mice. Statistical differences between sexes were tested with One-way ANOVA of log-transformed values with Holm-Šídák correction. (c) Fluorescence properties of *Salmonella* expressing the division-rate reporter $\text{TIMER}^{\text{bac}}$ when growing at defined rates *in vitro* (new representation of data reported in (3)). (d) $\text{TIMER}^{\text{bac}}$ -*Salmonella* fluorescence properties in spleen. Data for five to ten mice were pooled. (e) *Salmonella* spleen growth until day 4 post-infection after infection with about 1,000 CFU or about 50,000 CFU (High inoc.).

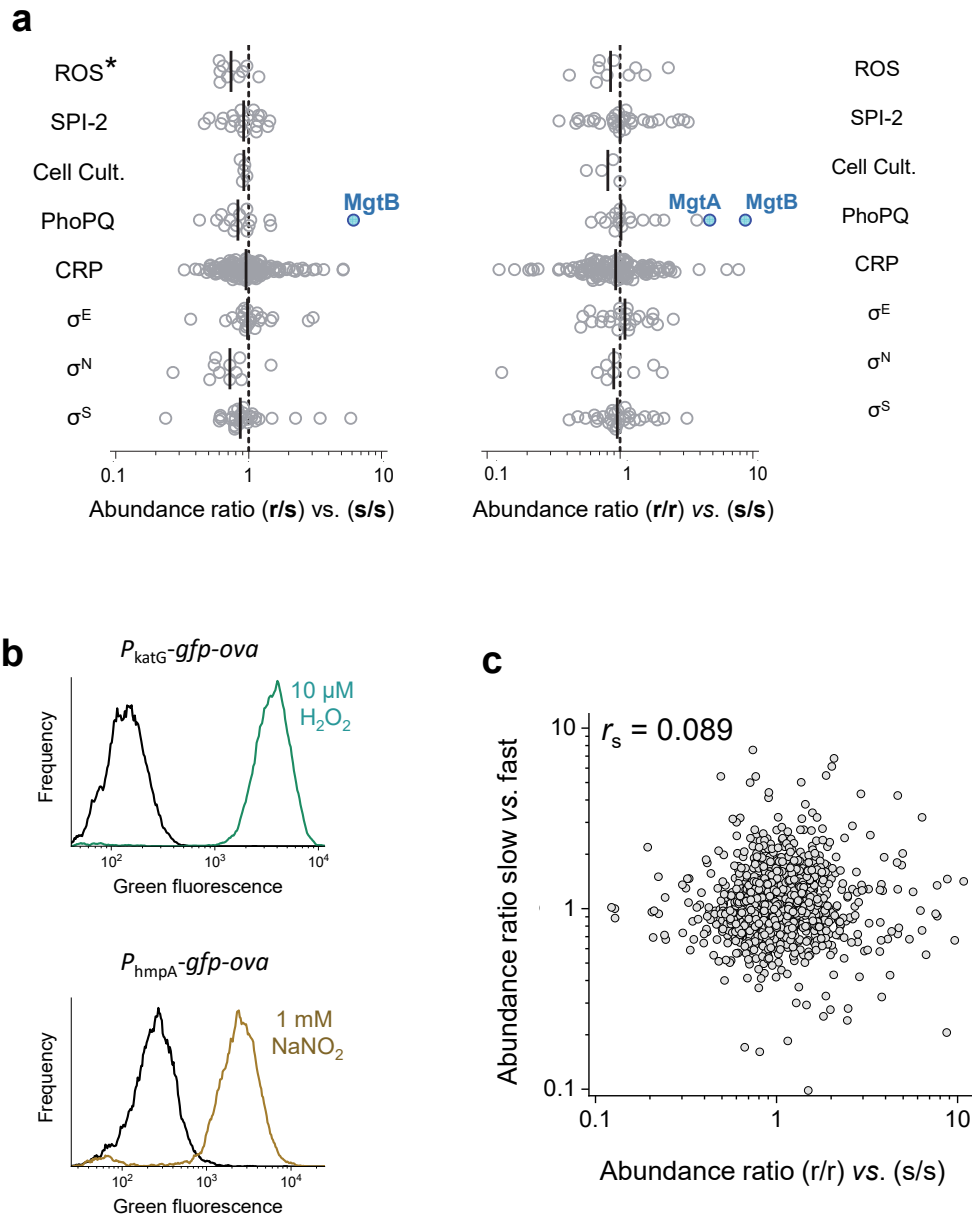


Fig. S3. Proteome analysis of *Salmonella* purified from different mouse genotypes. (a) Comparison of *Salmonella* protein abundances between *SLC11A1*^{s/s} and *SLC11A1*^{r/s} or *SLC11A1*^{r/r} mice for proteins associated with defense against reactive oxygen species (ROS), *Salmonella* pathogenicity island 2 (SPI-2), previous findings for SLC11A1-dependent proteins in cell culture infections (Cell Cult.), the PhoPQ regulon, the cAMP receptor protein CRP, or

various sigma factors of the RNA polymerase. Each circle represents one protein. The magnesium transporters MgtA and MgtB are part of the PhoPQ regulon and get additionally upregulated at low Mg^{2+} availability (*, $P < 0.05$; One-sample Wilcoxon signed rank test with Holm-Šídák correction for multiple testing). Carbon and nitrogen availability seemed to be similar based on the expression of target genes of σ^N , and σ^S . The same was true for extracytoplasmic stress based on σ^E targets. In addition to data shown in this figure, nitric oxide denitrosylase HmpA, a signature protein of Salmonella exposure to reactive nitrogen species (41) (abundance ratio (r/s) vs. (s/s), 1.2 ± 0.4 ; (r/r) vs. (s/s), 0.41 ± 0.2) and phosphate starvation markers PhoB (abundance ratios, 1.0 ± 0.3 , 0.8 ± 0.3) and PstS (1.1 ± 0.4 , 1.4 ± 0.4) were of comparable abundance. **(b)** Green fluorescence of *Salmonella* carrying promoter fusions P_{katG} -*gfp-ova* or P_{hmpA} -*gfp-ova* under *in vitro* control conditions without oxidative and nitrosative stress (black), or after exposure to hydrogen peroxide for 30 min, or nitrite at pH 5.5 for 60 min, respectively. **(c)** Comparison of protein abundance ratios in (r/s) vs. (s/s) mice, to protein ration in fast- vs. slow- -growing subsets in BALB/c (s/s) mice (data from (3); r_s , Spearman's rank correlation coefficient).

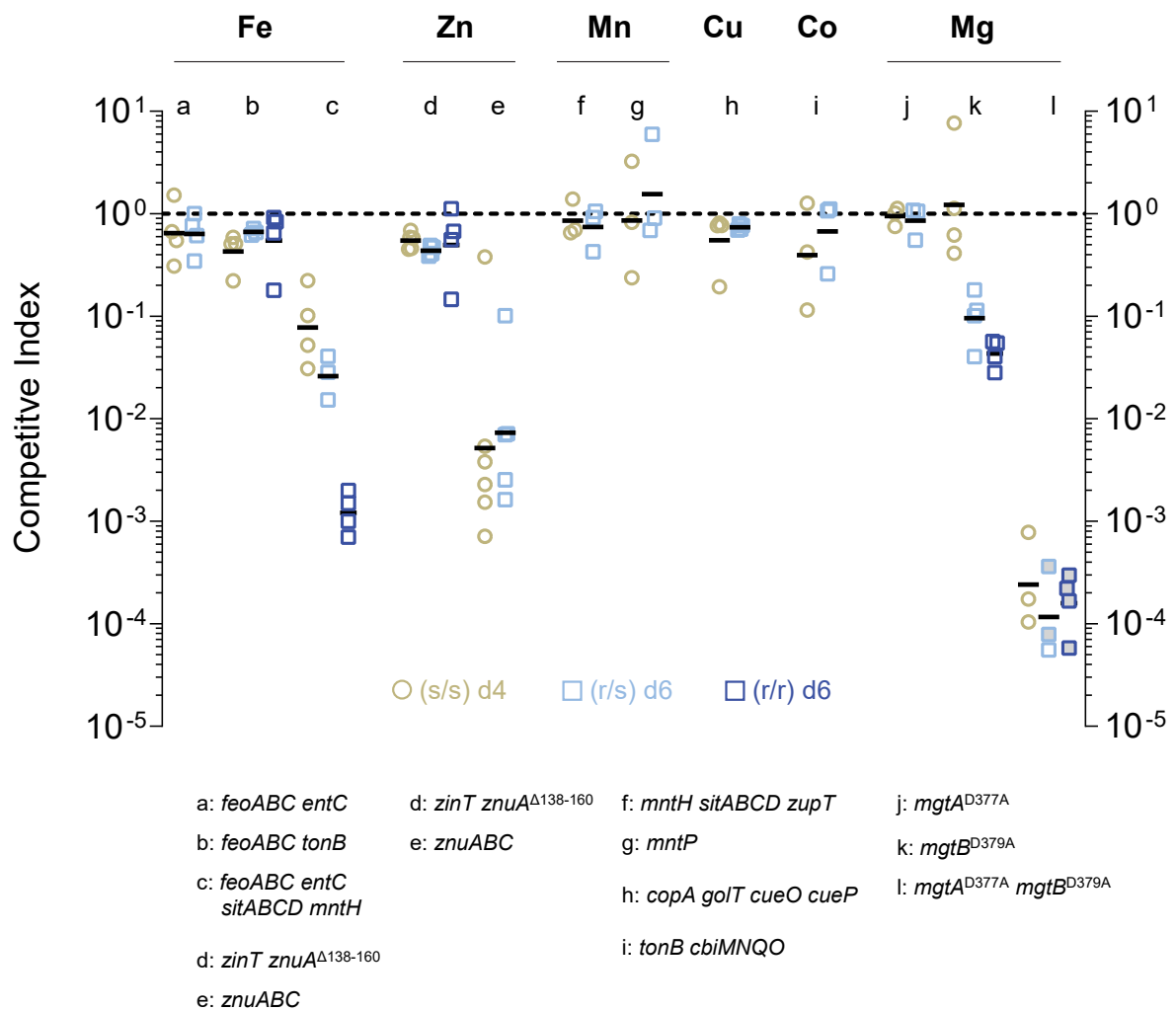


Fig. S4. Competitive indices of *Salmonella* mutants in *SLC11A1*^{s/s} (s/s), *SLC11A1*^{r/s} (r/s), or *SLC11A1*^{s/s} (r/r) mice. Mice were infected with mixtures of *Salmonella* wild-type and *Salmonella* mutants, and the competitive index for each mutant was calculated by dividing the output ratio (mutant/wildtype) by the input (mutant/wildtype). The dashed line at a value of 10⁰ represents wild-type fitness. Each circle represents a single mouse, the lines show the geometric means. Only mutants “b”, “c”, “d”, “k”, and “l” were tested in (r/r) mice. Data below the limit of detection (LOD) were substituted by LOD/√2 and are shown in grey. In contrast to relative fitness data shown in Fig. 3B,C, competitive indices increasingly diverge from 1 with ongoing divisions. Because *Salmonella* divided more slowly in (r/s) and (r/r) mice compared to (s/s) mice, CI values underestimated the mutant phenotypes in (r/s) and (r/r) mice relative to (s/s) mice.

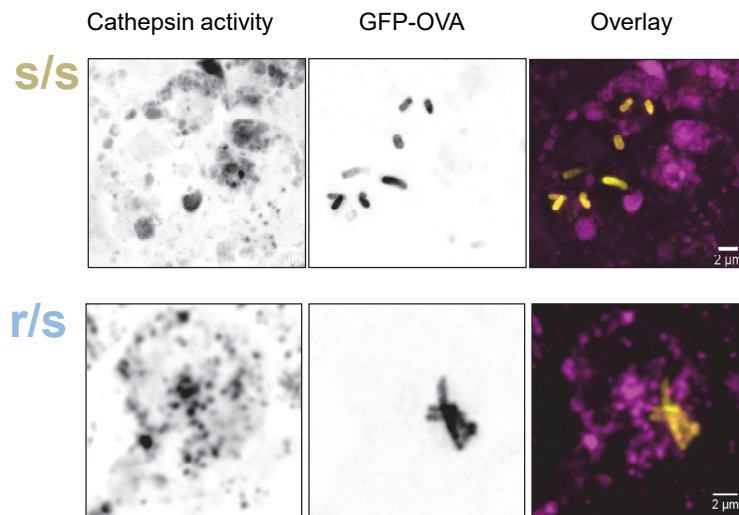
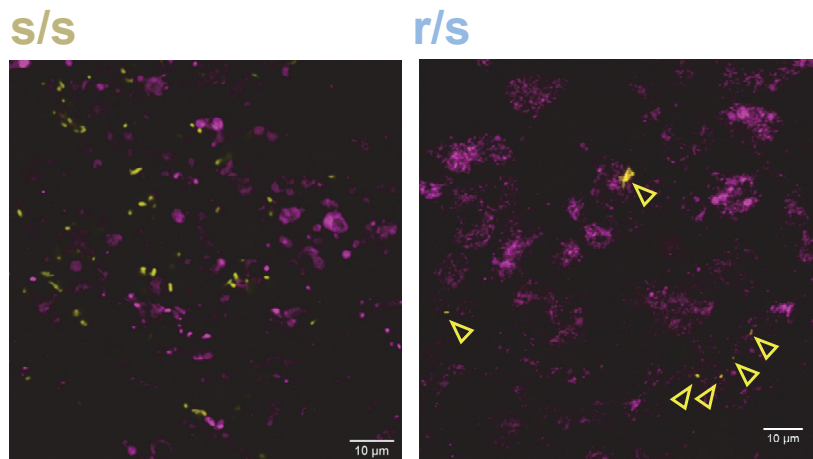


Fig. S5. Localization of cathepsin activities and *Salmonella* cells in spleen. Cathepsin activities (magenta, quenched-activity based probe iABP) and GFP/YPet fluorescence of *Salmonella* cells (yellow) in spleens of *SLC11A1*^{s/s} (s/s) and *SLC11A1*^{r/s} (r/s) mice. The upper panels show confocal micrographs of spleen cryosections. The lower panels show for each mouse genotype one infected cell with high cathepsin activities but no detectable co-localization, at higher magnification. Similar images were obtained for sections from three independently infected *SLC11A1*^{r/s} mice. Infected control mice with no iABP injection showed only faint background signals.

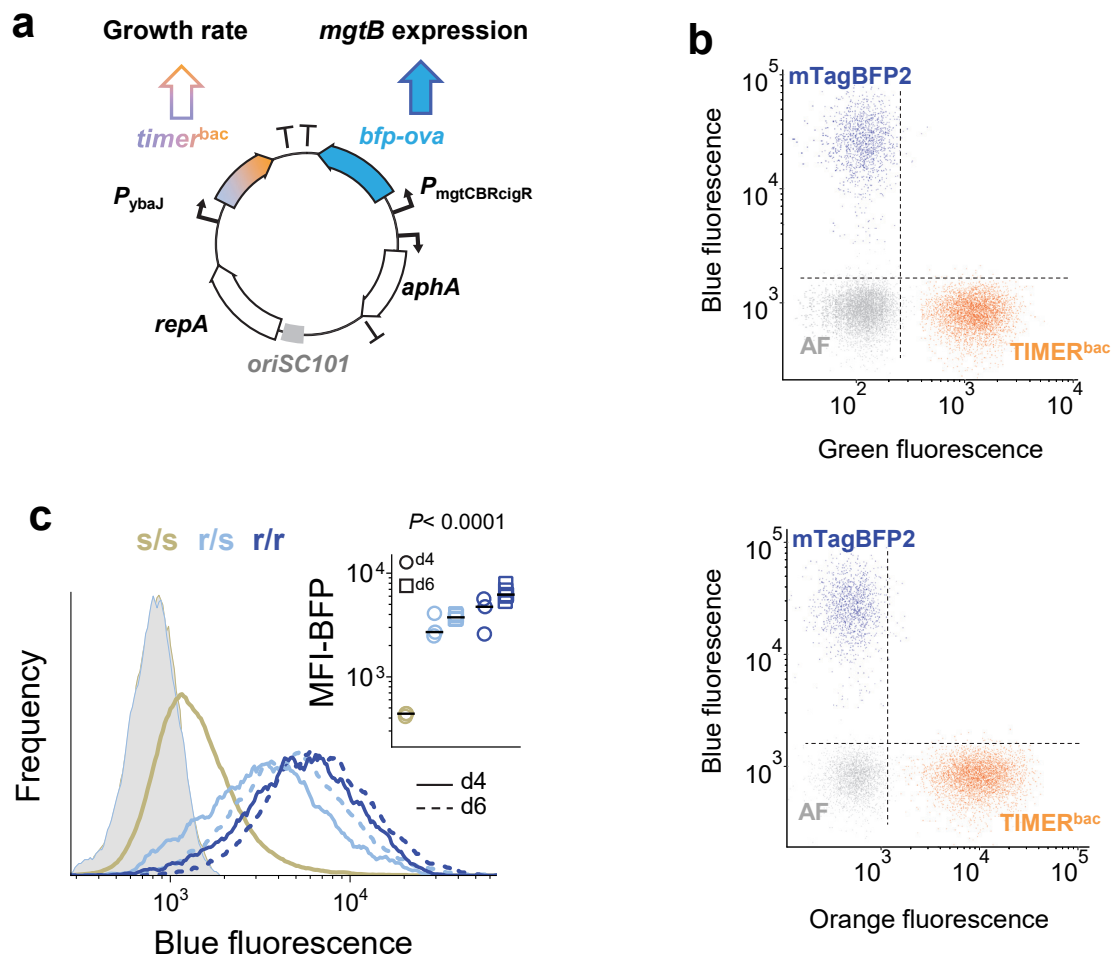


Fig. S6. Measurement of correlations between *Salmonella* replication and transporter expression. (a) Map of dual reporter plasmid (*timer^{bac}* codes for the growth rate reporter TIMER^{bac} (3); *bfp-ova* codes for a fusion of the blue fluorescent protein mTagBFP2 with an ovalbumin peptide that increases proteolysis of fluorescent proteins (4) for reporting current promoter activities with minimal contributions from previous expression; T, terminator; *aphA* confers resistance to kanamycin; *repA* codes for replicase recognizing the origin of replication, *oriSC101*). (b) Flow cytometry of *Salmonella* strains expressing mTagBFP2, TIMER^{bac}, or neither of the two fluorescent proteins (autofluorescence, AF) in spleen homogenates. The dashed lines represent upper limits of autofluorescence. mTagBFP2 and TIMER^{bac} have orthogonal fluorescence colors with no bleed-through in blue / green fluorescence channels

(upper panel) and blue / orange fluorescence channels (lower panel). (c) Blue fluorescence of *Salmonella* carrying the dual reporter plasmid shown in (a) in mice with different *SLC11A1* genotypes. The shaded histograms represent autofluorescence of *Salmonella* with no BFP expression. Histograms represent pooled data for three to five mice. The inset shows median BFP fluorescence intensities corrected for blue autofluorescence (MFI-BFP) with each symbol representing a single mouse (*P*-value for One-way ANOVA with test for linear trend). *Salmonella* stably maintained the plasmid during infection without detectable fitness impairment (< 1% plasmid loss after six days based on fluorescence of colonies grown *ex vivo* on non-selective medium). $P_{\text{mgtCBRcigR}}$ activity was 7.2 to 15 fold higher in (r/s) and (r/r) mice compared to (s/s) mice, consistent with 6.2 ± 2.7 and 8.7 ± 2.3 fold higher abundance of the MgtB protein in (r/s) and (r/r) mice, respectively (Fig. 2A).

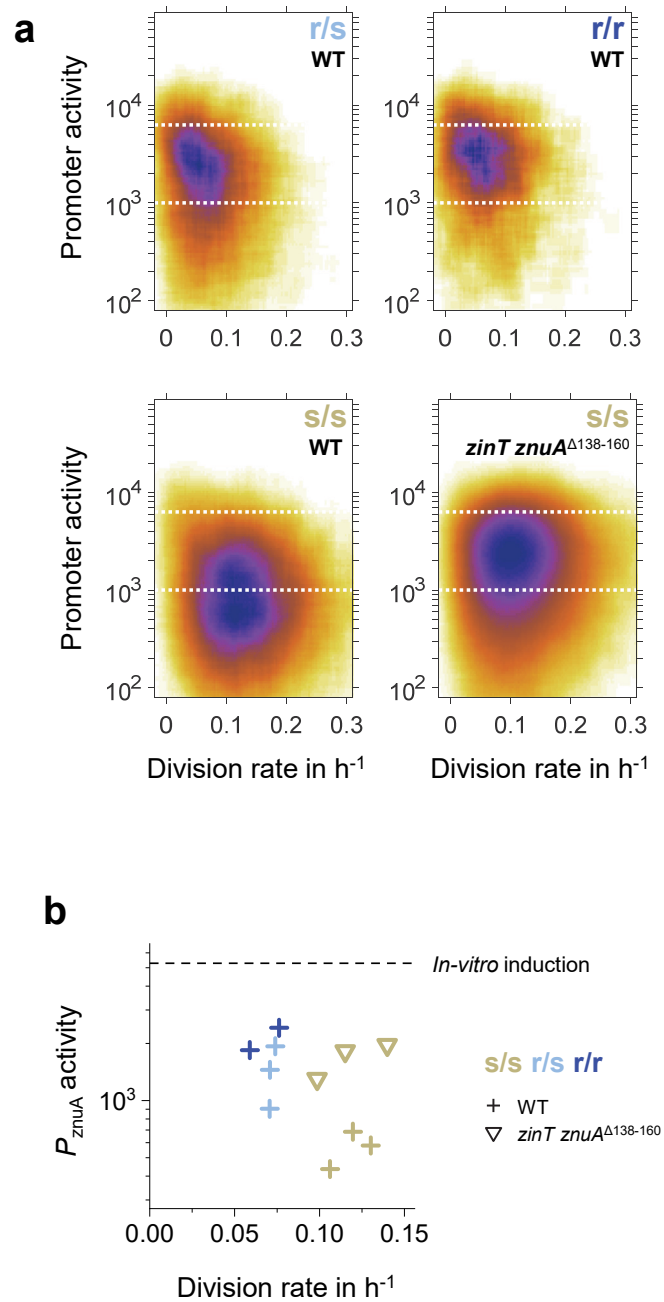


Fig. S7. Relation between *Salmonella* replication and expression of the zinc transporter gene *znuA*. (a) *Salmonella* division rates based on $\text{TIMER}^{\text{bac}}$ emission color, and P_{ZnuA} activities at day 4 post-infection. *znuA* encodes the crucial periplasmic Zn^{2+} -binding protein ZnuA (18), which is strongly induced by zinc starvation (48). The plots represent pooled data from two to

three mice. The dotted white lines represent the range that contains the majority of P_{znuA} activities in (r/s) and (r/r) mice. P_{znuA} activity was 2.5 ± 1.1 fold higher in (r/s) mice and 3.8 ± 1.1 fold higher in (r/r) mice compared to (s/s) mice, consistent with higher abundance of the ZnuA protein in (r/s) and (r/r) mice (2.4 ± 0.6 fold, 2.8 ± 0.5 fold; Fig. 2A). **(b)** Median division rates and P_{znuA} activities for *Salmonella* strain/mouse genotypes shown in (a). Each symbol represents a single mouse. Symbol shapes represent *Salmonella* genotype and symbol colors represent mouse genotype. *In-vitro* induction levels in lysogeny broth containing 100 μ M EDTA are shown for comparison. The zinc-uptake mutant *Salmonella zinT znuA* ^{Δ 138-160} (mutant “d”) had as high *znuA* expression levels in (s/s) mice (3.0 ± 0.9 fold), as wild-type *Salmonella* in (r/s) and (r/r) mice, but nevertheless maintained high division rates consistent with the only slight attenuation of this mutant (Fig. 3B,C) (17, 18).

Transporter	References	Fe	Zn	Mn	Co	Mg	comment
TBDT	(49)	0.5 nM					ferri-siderophore
FeoABC	(50)	0.5 μ M					
SitABCD	(51)	3 μ M		0.1 μ M			
MntH	(52)	25-50 μ M		0.1 μ M			at pH 5.5
ZinT-ZnuABC	(53)		< 20 nM				
ZupT	(54)		0.71 μ M	1.16 μ M	0.91 μ M		
BtuB	(55)				16 nM		cob(I)alamin
CorA	(56, 57)				30 μ M	15 μ M	
MgtA	(57)					29 μ M	
MgtB	(57)					6 μ M	

Table S1. Apparent metal affinities of divalent cation transporters (as shown in Fig. 3A).