**Figure S1**



**Figure S1.** Minimal inhibitory concentration of Gentamicin against *E. coli* ATCC 25922 under NS and HS conditions (means ± s.e.m; n = 4; Mann Whitney test; \* p< 0.05).

**Figure S2**



**Figure S2.** Intracellular Ca2+ and K+ levels upon HS exposure. (**A**) Intracellular Ca2+ levels were assessed in Fura-2-loaded and *E. coli*-infected RAW264.7 MΦ by ratiometric live cell imaging. The arrow indicates the addition of 40 mM NaCl to infected cells (means ± s.e.m; n = 6). When indicated, 1 µg/ml Iono (ionomycin) was added to trigger Ca2+-efflux as control. (**B**) As in (A), but K+ levels were assessed in PBFI-loaded and *E. coli*-infected RAW264.7 MΦ (means ± s.e.m; n = 4 - 6). When indicated, ATP + Iono (25 mM ATP + 10 µg/ml ionomycin) was added to induce K+-efflux as control.

**Figure S3**



**Figure S3.** HS increases autophagy in *E. coli*-infected MΦ. (**A**) RAW264.7 MΦ were pretreated ± bafilomycin A1 and infected ± HS for ½ h. Densitometry of immunoblots for MAP1LC3B and loading controls (ACT or HSP90; n = 8; paired Student’s t tests; \* p < 0.05). (**B**)As (A) but for 2 h. Densitometry of immunoblots stained for SQSTM1 and loading controls (ACT or HSP90; n = 3; paired Student’s t test; \* p < 0.05). (**C**) As (A) but WT and *cybb*-/- BMDM were used. Densitometry of immunoblots stained for MAP1LC3B and loading controls (ACT or HSP90; n = 6; paired Student’s t tests; \* p < 0.05).

**Figure S4**

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**Figure S4**. Autophagy and lysosomal acidification are required for high Na+-increased antibacterial activity. (**A**) Infected control and *atg7* cKOBMDM were treated ± HS for 2 h. Densitometry of immunoblots stained for ATG7 and loading controls (ACT or HSP90; n = 5; paired Student’s t tests; \* p < 0.05). (**B**) BMDM were infected as described in Fig. 1F. Where indicated, the cathepsin inhibitor E-64d (10 µM) was added prior infection. CTSB activity was measured (means ± s.e.m; Student’s t test + Welch correction and Mann Whitney test; n = 6; \* p < 0.05). (**C**) As in (A), but intracellular *E. coli* in CFU relative to mean CFU under NS conditions (means ± s.e.m; n = 12; Student’s t test + Welch correction; \* p < 0.05).

**Figure S5**



**Figure S5.** NFAT5 is required for HS-facilitated targeting of intracellular *E. coli* to autolysosomes. (**A**) ns siRNA- or *Nfat5*-specific siRNA-treated RAW264.7 MΦ were infected and stimulated ± HS for ½ h. Densitometry of immunoblots stained for NFAT5 and loading controls (ACT or HSP90; n = 6; Wilcoxon signed rank tests; \* p < 0.05). (**B**)As (A)but for 2 h.One representative blot and densitometry of several immunoblots stained for ATG7 and loading controls (ACT or HSP90; n = 5; paired Student’s t tests; \* p < 0.05).

**Figure S6**

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**Figure S6.** Blunted AKT and MTOR activation does not account for HS-augmented antibacterial activity. (**A**) RAW264.7 MΦ were pretreated ± Torin1 and infected with *E. coli* ± HS for ½ h. Densitometry of immunoblots for p-AKT and AKT (n = 7; paired Student’s t tests; \* p < 0.05). (**B**) As (A) but densitometry of immunoblots for MAP1LC3B and loading controls (ACT or HSP90; n = 5; paired Student’s t tests; \* p < 0.05). (**C**) As (A) but for 2 h. Densitometry of immunoblots stained for NFAT5 and loading controls (ACT or HSP90; n = 5; paired Student’s t tests; \* p < 0.05).

**Figure S7**



**Figure S7.** The presence of HIF1A is required for HS-triggered autophagy induction and antibacterial activity.(**A**)RAW264.7 MΦ were infected and stimulated ± HS for 2 h. Densitometry of immunoblots stained for HIF1A and loading controls (ACT or HSP90; n = 6; Wilcoxon signed rank test; \* p < 0.05). (**B**) As in Fig. 1A, but *Pgk1* and *Slc2a1* mRNA levels were quantified (means ± s.e.m; n = 6; Student’s t test or Mann Whitney test, \* p < 0.05). (**C**) After treating RAW264.7 MΦ with ns siRNA or *Hif1a* siRNA*,* *Hif1a* and *Bnip3* mRNA levels were quantified 2 h after infection ± HS (means ± s.e.m; n = 6; Student’s t test; \* p < 0.05). (**D**) ns siRNA- or *Hif1a*-specific siRNA-treated RAW264.7 MΦ were infected and stimulated ± HS for 4 h. Densitometry of immunoblots for HIF1A and loading controls (ACT or HSP90; n = 4; paired Student’s t test; \* p < 0.05). (**E**) ns siRNA- or *Hif1a*-specific siRNA-treated RAW264.7 MΦ were infected and stimulated ± HS for ½ h. Densitometry of immunoblots stained for MAP1LC3B and loading controls (ACT or HSP90; n = 5; paired Student’s t test; \* p < 0.05).

**Figure S8**

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**Figure S8.** Relationship of HIF1A and NFAT5 upon exposure to HS. (**A**)Quantification of *Nfat5* mRNA levels in *E. coli*-infected MΦ ± HS, which were either treated with non-silencing (ns) siRNA or *Hif1a*-specific siRNA at 2 h post infection (n = 13, Student’s t test or Mann-Whitney U test; \* p < 0.05). (**B**)As in (A), but quantification of NFAT5 and ACT protein levels was performed after 4 h (n = 4, paired Student’s t test; \* p < 0.05). (**C**)Quantification of *Hif1a* mRNA levels in MΦ ± HS at 2 h post *E. coli*-infection, which were either pretreated with ns siRNA or *Nfat5*-specific siRNA (n = 9; Mann-Whitney U test, \* p < 0.05). (**D**) Cells were treated as in (C), but quantification of HIF1A protein levels was performed (n = 6; paired Student’s t test or Wilcoxon signed rank test; \* p < 0.05. (**E**) Cells were treated as in (C), but with an additional conditions using 50 µM DMOG. At 4 h post infection, protein levels of HIF1A and ACT were determined. (**F**) Cells were treated as in (E), but bacterial survival in these cells was analyzed 2 h post infection (n = 18, ANOVA and Bonferroni’s multiple comparison test, \* p < 0.05).