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Supplemental information

Mitochondrial respiration restricts *Listeria monocytogenes* infection by slowing down host cell receptor recycling

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Figure S1

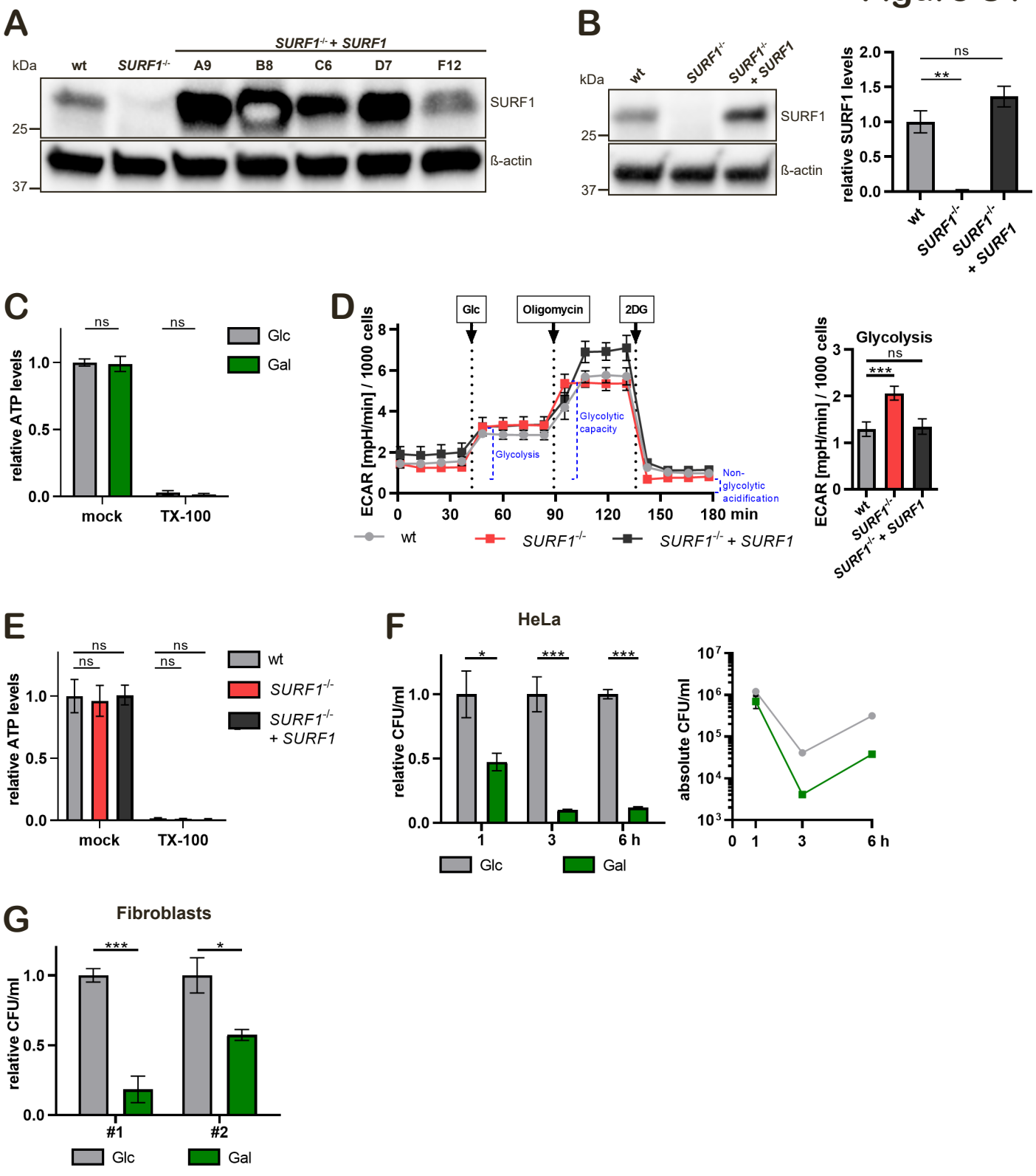


Figure S1: Changes in the cellular energy production pathways affect *L. monocytogenes* infection. Related to Figure 1.

(A) Immunoblot analysis of SURF1 protein levels in HCT116 wt, *SURF1*$^{-/-}$ cells as well as several complemented *SURF1*$^{-/-}$ clones. ß-actin protein levels were used as loading control.

(B) Immunoblot analysis of SURF1 protein levels in HCT116 wt, *SURF1*$^{-/-}$ and *SURF1*$^{-/-}$ + *SURF1* cells. ß-actin protein levels were used as loading control. Data represent mean ± SEM of three independent experiments and statistical significance was determined by one-way ANOVA with Dunnett's post hoc test (ns, not significant; **, $P < 0.01$). Note that complemented *SURF1*$^{-/-}$ cells express slightly more SURF1 than wt cells (136 %).

(C) Cellular ATP levels in HCT116 Glc and Gal cells were quantified using the ATPlite luminescence assay kit. Cells treated with Triton X-100 (TX-100) served as negative control. Three independent experiments were performed and data from one representative experiment with ≥ 4 biological replicates is displayed as mean ± SD. Statistical significance was determined by two-tailed t-tests (ns, not significant).

Figure S1

(D) Extracellular acidification rate (ECAR, in mpH/min) of HCT116 wt, *SURF1*$^{-/-}$, and *SURF1*$^{-/-}$ + *SURF1* cells, monitored in a Seahorse XFe96 analyzer. Three independent experiments were performed and data from one representative experiment with six biological replicates per condition are shown as mean ± SD for each time point. The glycolysis rates were statistically evaluated by one-way ANOVA with Dunnett's post hoc test (ns, not significant; ***, $P < 0.001$).
(E) Cellular ATP levels in HCT116 wt, *SURF1*$^{-/-}$, and *SURF1*$^{-/-}$ + *SURF1* cells were quantified using the ATPlite luminescence assay kit. Treatment of the cells with Triton X-100 (TX-100) served as negative control. Three independent experiments were performed and data from one representative experiment with ≥ 4 biological replicates are displayed as mean ± SD. Statistical significance was determined by one-way ANOVA with Dunnett's post hoc test (ns, not significant).
(F) Quantification of adhered and intracellular (1 h) and intracellular (3-6 h) bacteria in HeLa Glc and Gal cells upon infection with GFP-expressing wt *L. monocytogenes* EGD (MOI 20). The left panel shows relative values, which have been normalized to HeLa Glc cells, and the right panel shows CFU/mL. Three independent experiments were performed and data from one representative experiment with three biological replicates are shown as mean ± SD. Statistical significance was calculated by multiple two-tailed t-tests and evaluated by the False Discovery Rate approach of Benjamini, Krieger and Yekutieli, with Q = 1 % (*, $P < 0.05$; ***, $P < 0.001$).
G) Intracellular bacterial load in primary fibroblasts maintained in Glc or Gal medium, which were infected with wt *L. monocytogenes* EGDe (MOI 20), at 18 h post infection. Three independent experiments were performed and data from one representative experiment with three biological replicates are shown as mean ± SD. For each time point, values have been normalized to fibroblasts growing in Glc medium. Statistical significance was calculated by a two-tailed t-test (*, $P < 0.05$; ***, $P < 0.001$).

Figure S2

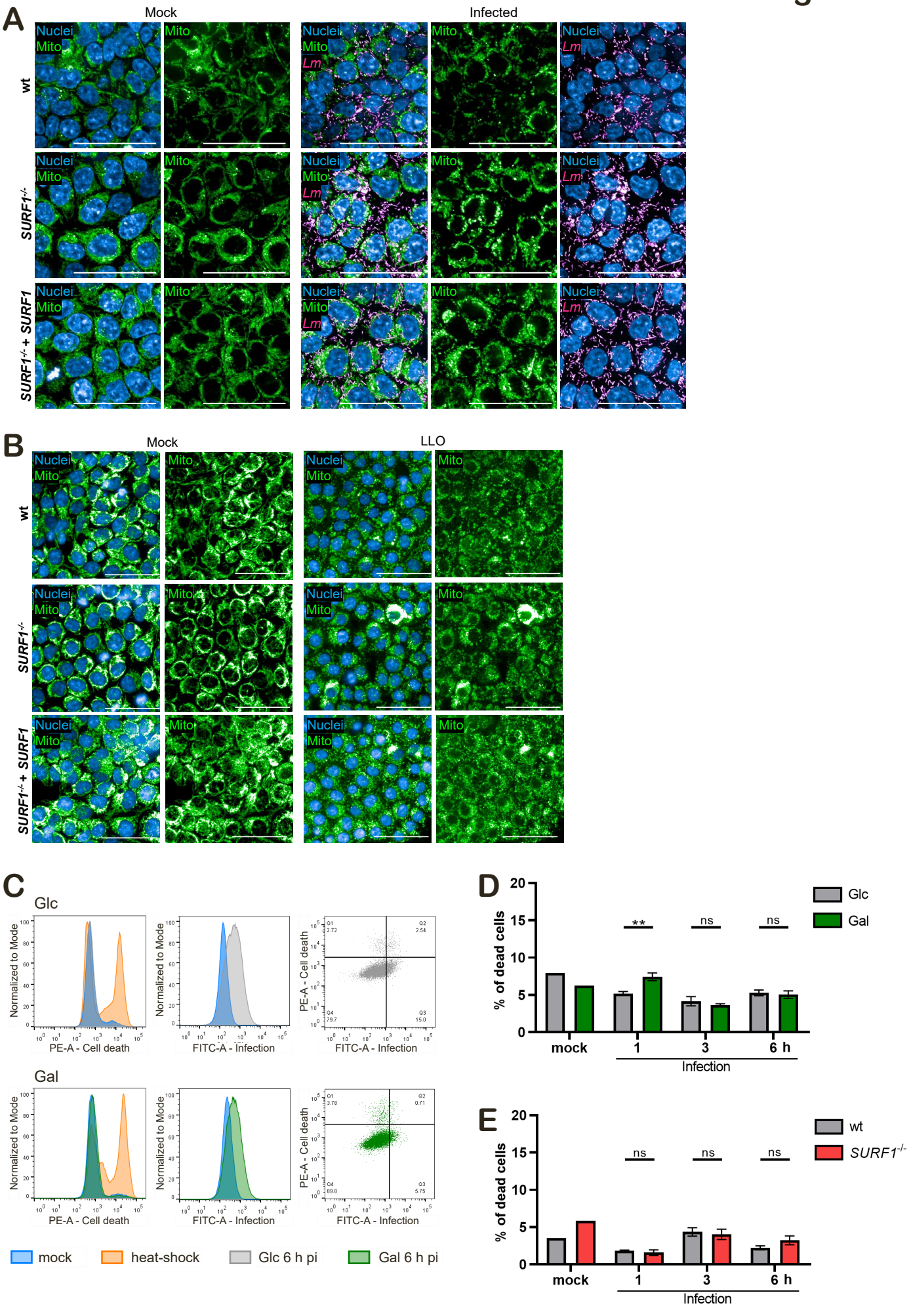

A
Mock
Infected
wt
SURF1-/-
SURF1-/- + SURF1
Nuclei
Mito
Lm
B
Mock
LLO
wt
SURF1-/-
SURF1-/- + SURF1
Nuclei
Mito
C
Glc
Gal
Normalized to Mode
PE-A - Cell death
FITC-A - Infection
PE-A - Cell death
mock
heat-shock
Glc 6 h pi
Gal 6 h pi
D
% of dead cells
Glc
Gal
**
ns
ns
mock
1
3
6 h
Infection
E
% of dead cells
wt
SURF1-/-
ns
ns
ns
mock
1
3
6 h
Infection

Figure S2

Figure S2: Manipulation of mitochondrial respiration does not impact mitochondrial morphology or cell death.

Related to Figure 1.

(A) Representative confocal images of the mitochondrial network of uninfected (mock) and infected HCT116 wt, *SURF1*$^{-/-}$, and *SURF1*$^{-/-}$ + *SURF1* cells. Cells were (mock) infected with GFP-expressing wt *L. monocytogenes* EGD (Lm, MOI 20, displayed in pink) for 1 h, followed by treatment with 20 µg/mL gentamicin. Mitochondria were stained with Mitotracker Deep red (green) and nuclei with NucBlue (blue). Scale bar, 50 µm.

(B) Representative confocal images of the mitochondrial network of HCT116 wt, *SURF1*$^{-/-}$, and *SURF1*$^{-/-}$ + *SURF1* cells treated without (mock) or with 6 nM recombinant LLO. Mitochondria were stained with Mitotracker Deep red (green) and nuclei with NucBlue (blue). Scale bar, 50 µm.

(C) Representative flow cytometry analysis of infection and cell death in HCT116 Glc and Gal cells infected with GFP-expressing *L. monocytogenes* EGDe (MOI 20) for the indicated time points and stained with the LIVE/DEAD Fixable Red Dead Cell Stain Kit. Histograms display cell death (PE-A) or infection (FITC-A) levels and dot plots show the distribution of infected and dead cells at 6 h post infection.

(D-E) Percentage of dead HCT116 Glc and Gal cells (D, same experiment as in C) and wt and *SURF1*$^{-/-}$ cells (E) upon infection with GFP-expressing *L. monocytogenes* EGDe as quantified by flow cytometry. Three (D) or two (E) independent experiments were performed and one representative experiment with three biological replicates is shown. Statistical significance was determined by multiple t-tests and evaluated by the False Discovery Rate approach of Benjamini, Krieger and Yekutieli, with Q = 1 % (ns, not significant; **, $P < 0.01$).

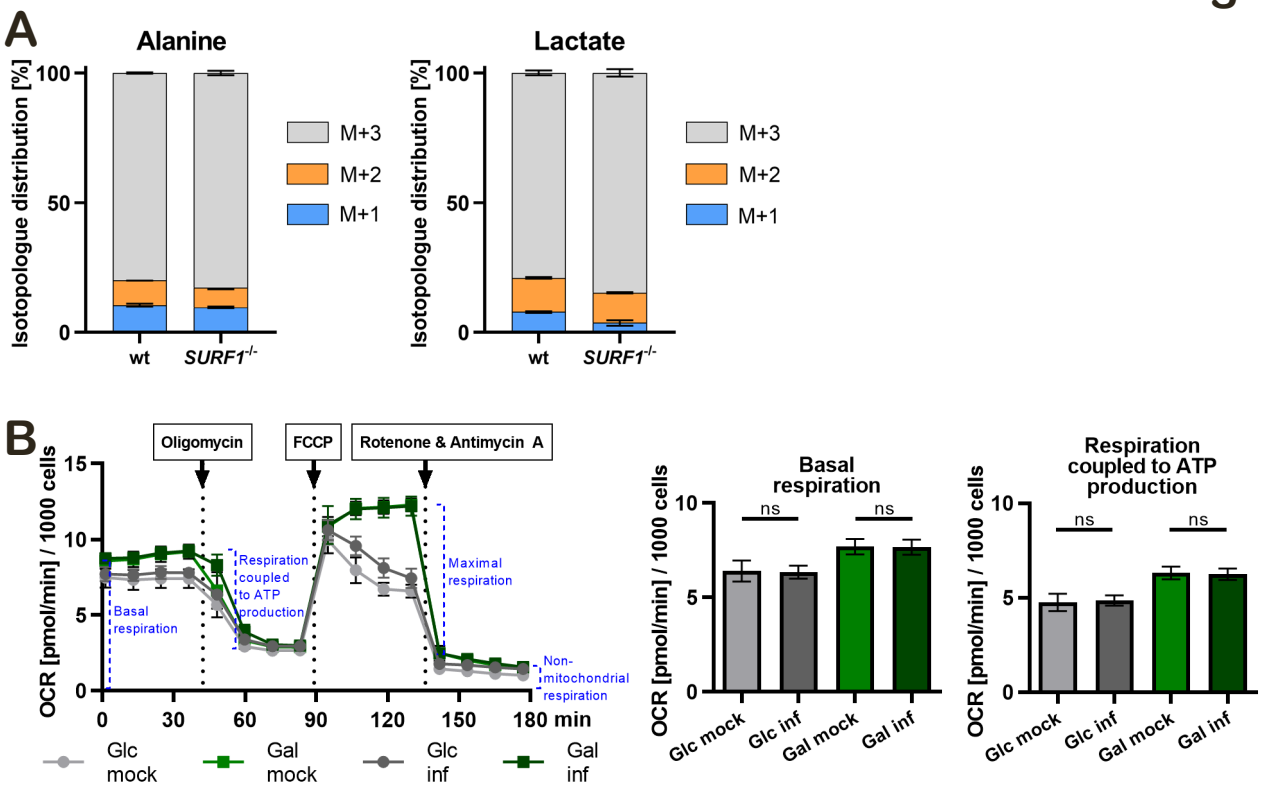
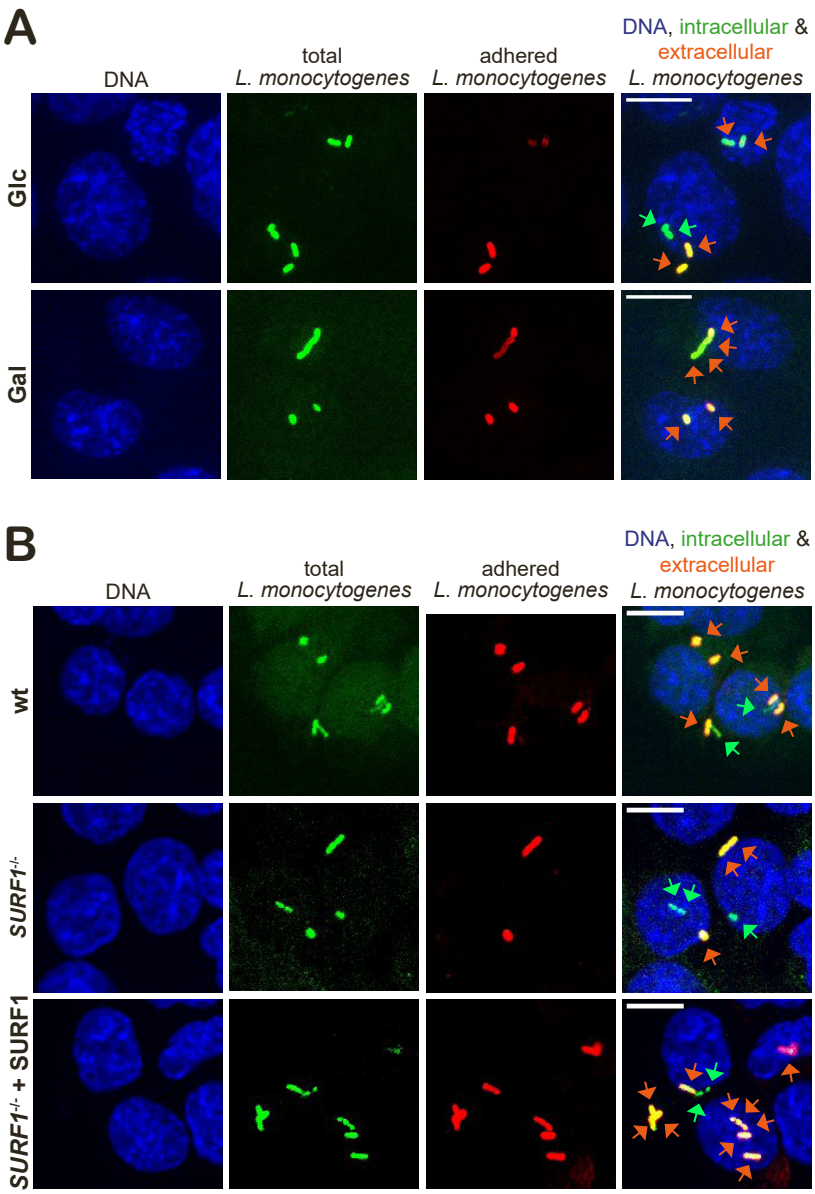
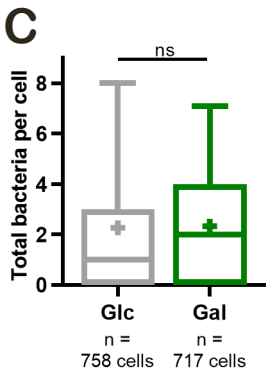
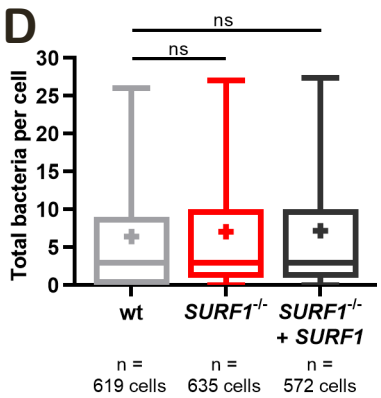


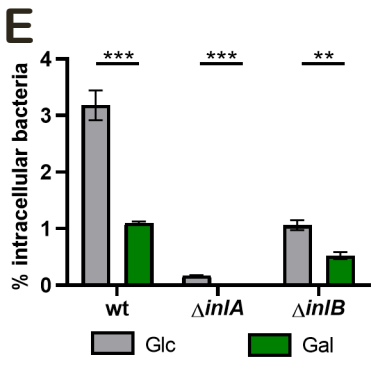
Figure S3: SURF1 ablation enhances the glycolytic activity.
Related to Figure 2.
(A) Relative ^{13}C isotopologue abundance in alanine and lactate isolated from the cytosol of HCT116 wt and *SURF1*$^{-/-}$ cells after growth on [U-$^{13}C_6$]glucose as quantified by GC-MS. Data is from the same experiments shown in Figure 2C, and displayed as mean ± SD (cumulative biological and technical errors). M+X indicates an isotopologue containing X ^{13}C-atoms.
(B) Oxygen consumption rate (OCR, in picomoles per minute) of uninfected (mock) and infected (inf) HCT116 Glc and Gal cells monitored in a Seahorse XFe96 analyzer. Cells were (mock) infected with wt *L. monocytogenes* EGDe (MOI 20) for 6 h. Two independent experiments were performed and data from one representative experiment with six biological replicates per condition are shown as mean ± standard deviation (SD) for each time point. The rates of basal respiration, and maximal respiration were statistically evaluated by two-tailed t-tests (ns, not significant).

Figure S4


A
DNA
total
L. monocytogenes
adhered
L. monocytogenes
DNA, intracellular &
extracellular
L. monocytogenes
Glc
Gal
B
DNA
total
L. monocytogenes
adhered
L. monocytogenes
DNA, intracellular &
extracellular
L. monocytogenes
wt
SURF1-/-
SURF1-/- + SURF1


C
ns
Total bacteria per cell
0
2
4
6
8
Glc
Gal
n =
758 cells
n =
717 cells


D
ns
ns
Total bacteria per cell
0
5
10
15
20
25
30
wt
SURF1-/-
SURF1-/-
+ SURF1
n =
619 cells
n =
635 cells
n =
572 cells


E
***
***
**
% intracellular bacteria
0
1
2
3
4
wt
ΔinlA
ΔinlB
Glc
Gal

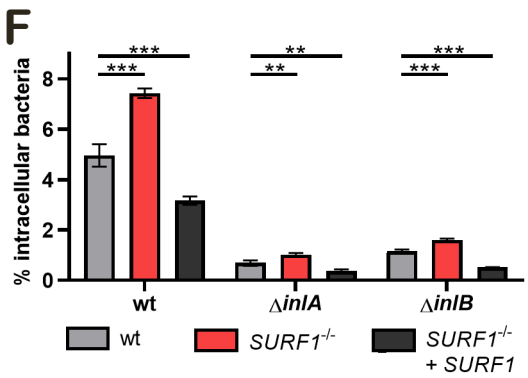

F
***
***
**
**
***
***
% intracellular bacteria
0
2
4
6
8
wt
ΔinlA
ΔinlB
wt
SURF1-/-
SURF1-/-
+ SURF1

Figure S4

Figure S4: *L. monocytogenes* entry is impaired by mitochondrial respiration.
Related to Figure 3.
(A-B) Representative immunofluorescence analysis of the differential bacterial staining for adhered and intracellular bacteria in HCT116 Glc and Gal cells (A) and HCT116 wt, *SURF1*$^{-/-}$ and *SURF1*$^{-/-}$ + *SURF1* cells (B). Cells were infected with GFP-expressing wt *L. monocytogenes* EGD (MOI 20, 1 h); extracellular (adhered) bacteria were stained in red (anti-R11) and nuclei in blue. In the overlay, green arrows indicate intracellular bacteria and orange arrows indicate adhered bacteria. Scale bar, 10 µm.
(C-D) Quantification of *L. monocytogenes* EGD (MOI 20, 1 h) adhered and internalized by HCT116 Glc and Gal cells (C) or HCT116 wt, *SURF1*$^{-/-}$ and *SURF1*$^{-/-}$ + *SURF1* cells (D) by immunofluorescence. Results are representative of four (C) and three (D) independent experiments and are displayed as box and whiskers plots with absolute numbers of bacteria per cell (with n > 570 cells per condition). Statistical significance was determined using a two-tailed Mann-Whitney test (C) or a Kruskal-Wallis test followed by Dunn's multiple comparisons test (D) (ns, not significant).
(E-F) Quantification of intracellular *L. monocytogenes* EGD in HCT116 Glc and Gal cells (E) or HCT116 wt, *SURF1*$^{-/-}$ and *SURF1*$^{-/-}$ + *SURF1* cells (F) after infection with wt, InlA-deficient (Δ*inlA*) or InlB-deficient (Δ*inlB*) bacterial strains for 1 h. Three independent experiments were performed and data from one representative experiment with three biological replicates are shown as % intracellular bacteria. Statistical significance was determined by two-tailed t-tests (E) or one-way ANOVAs with Dunnett's post hoc test (F) (ns, not significant; **, $P < 0.01$; ***, $P < 0.001$).

Figure S5

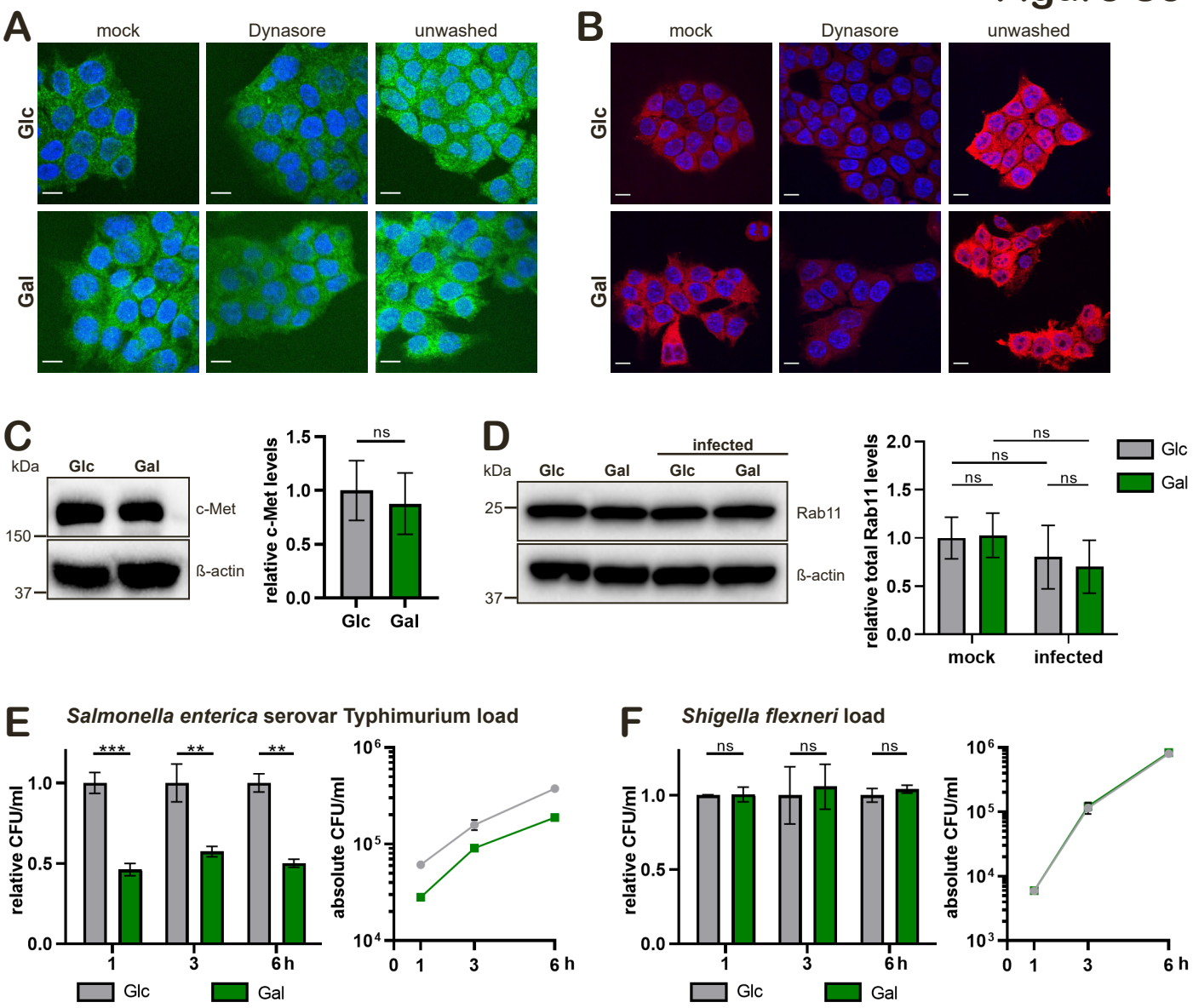


Figure S5: The host cell metabolism impacts endocytic recycling.

Related to Figure 4.

(A-B) Representative immunofluorescence analysis of total (unwashed) and intracellular TfR (A) or c-Met (B) at 3 min incubation with the respective antibodies and subsequent washing with high-salt, low-pH buffer (mock). As a negative control, cells were treated with Dynasore. TfR is displayed in green, c-Met in red, and nuclei in blue. Scale bar, 10 µm.

(C) Immunoblot analysis of c-Met levels in HCT116 Glc and Gal cells. ß-actin protein levels were used as loading control. Data represent mean ± SEM of three independent experiments and statistical significance was determined by a two-tailed t-test (ns, not significant).

(D) Immunoblot analysis of Rab11 levels in uninfected (mock) and infected HCT116 Glc and Gal cells, which were (mock) infected with wt *L. monocytogenes* EGDe (MOI 20) for 6 h. ß-actin protein levels were used as loading control. Data represent mean ± SEM of three independent experiments and statistical significance was determined by one-way ANOVA with Dunnett's post hoc test (ns, not significant).

(E-F) Intracellular bacterial load in HCT116 Glc and Gal cells infected with wt *Salmonella enterica* serovar Typhimurium 12023 (E, MOI 50) or *Shigella flexneri* M90T (F, MOI 20). The left panel shows values for Gal cells relative to Glc cells, and the right panel shows the absolute quantification (CFU/mL), for each time point. Two independent experiments were performed, and for both panels one representative experiment with three biological replicate is shown as mean ± SD. Statistical significances were calculated by multiple t-tests and evaluated by the False Discovery Rate approach of Benjamini, Krieger and Yekutieli, with Q = 1 % (ns, not significant; **, $P < 0.01$; ***, $P < 0.001$).