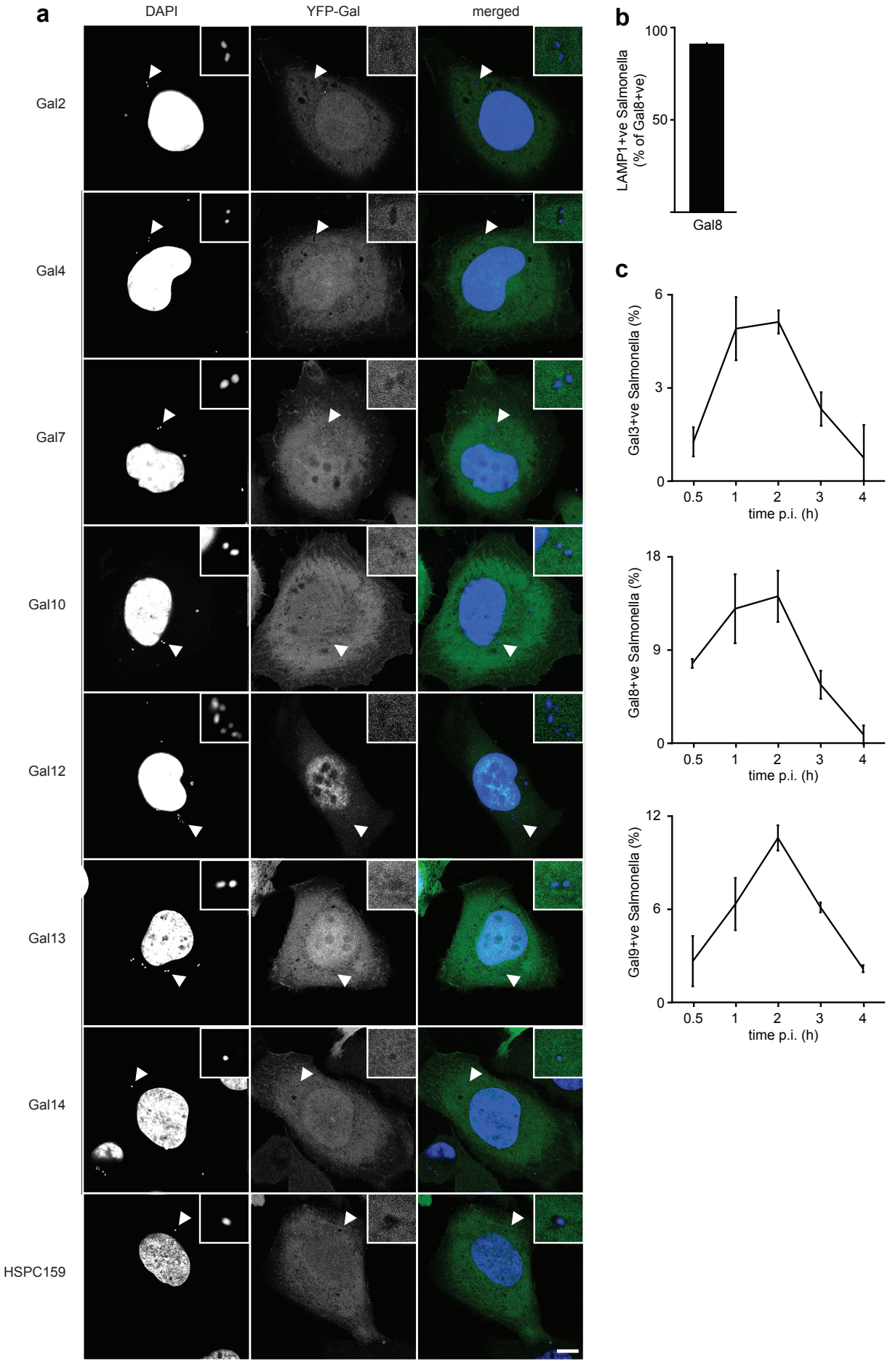
Supplementary Figure 1. Analysis of galectin colocalization with *S*.Typhimurium.

A) Confocal microphotographs of HeLa cells expressing the indicated YFP-tagged galectins at 1h post infection with *S*.Typhimurium.

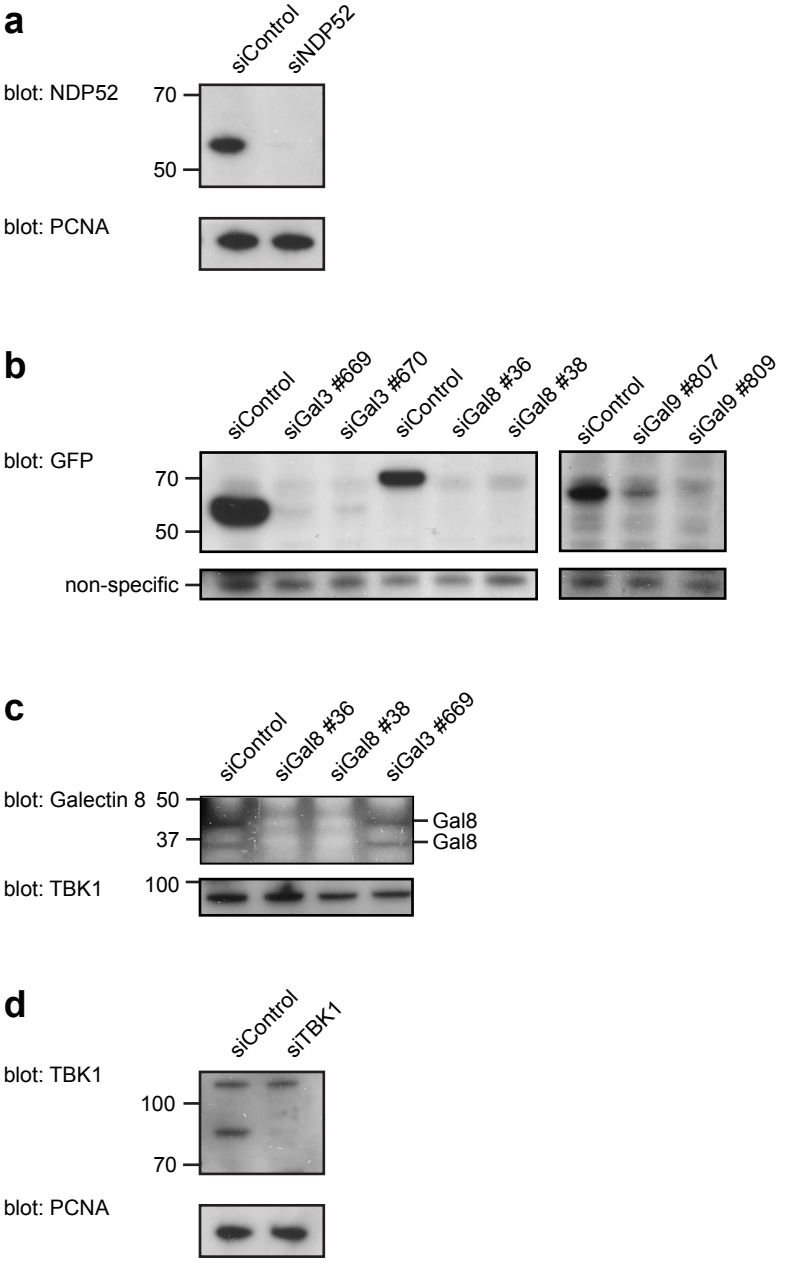
B) Colocalization of LAMP1 with galectin-8-positive bacteria in HeLa cells stably expressing YFP-tagged galectin-8, infected with *S*.Typhimurium and stained with LAMP1 antibody 1h after infection. Mean and s.d. of triplicate HeLa cultures, >200 bacteria were counted per coverslip.

C) Kinetic analysis of galectin recruitment in HeLa cells stably expressing the indicated YFP-tagged galectins and infected with *S*.Typhimurium. Percentage of YFP-positive bacteria were counted by microscopy at the indicated time points. Mean and s.d. of duplicate HeLa cultures. >100 bacteria counted per coverslip.


a
DAPI
YFP-Gal
merged
Gal2
Gal4
Gal7
Gal10
Gal12
Gal13
Gal14
HSPC159
b
LAMP1+ve Salmonella (% of Gal8+ve)
100
50
Gal8
c
Gal3+ve Salmonella (%)
6
3
0
0.5
1
2
3
4
time p.i. (h)
Gal8+ve Salmonella (%)
18
9
0
0.5
1
2
3
4
time p.i. (h)
Gal9+ve Salmonella (%)
12
6
0
0.5
1
2
3
4
time p.i. (h)

Supplementary Figure 2. Characterization of siRNAs

Protein levels in HeLa cells after treatment with the indicated siRNAs for 3 days. Lysates of HeLa cells were probed with antibodies against (A) endogenous NDP52, (B) against GFP fused to the indicated galectins, (C) against endogenous galectin-8, and (D) against endogenous TBK1. Lower panels, loading controls.

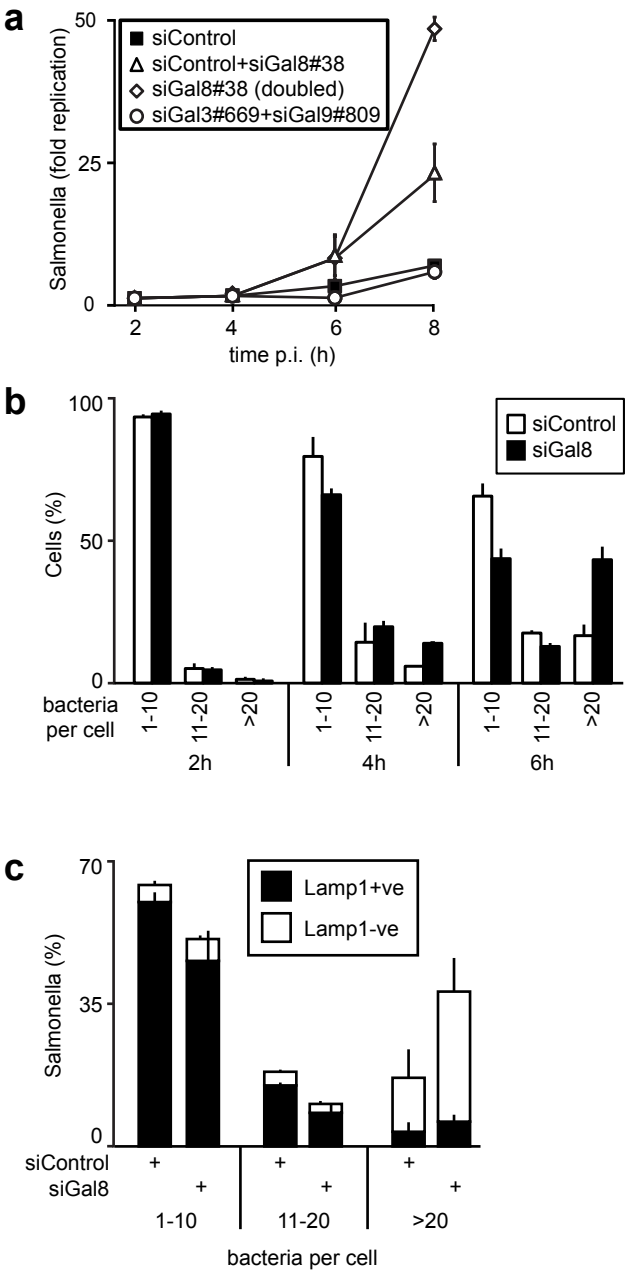

a
siControl
siNDP52
blot: NDP52
70
50
blot: PCNA
b
siControl
siGal3 #669
siGal3 #670
siControl
siGal8 #36
siGal8 #38
siControl
siGal9 #807
siGal9 #809
blot: GFP
70
50
non-specific
c
siControl
siGal8 #36
siGal8 #38
siGal3 #669
blot: Galectin 8
50
37
Gal8
Gal8
blot: TBK1
100
d
siControl
siTBK1
blot: TBK1
100
70
blot: PCNA

Supplementary Figure 3. Galectin-8 depletion causes hyperproliferation of *S*.Typhimurium in a Lamp1-negative compartment.

A) Lack of synergism between galectin-3 and galectin-9. Fold replication of *S*.Typhimurium in HeLa cells transfected with the indicated siRNAs. At the indicated time points after infection, cells were lysed and bacteria counted on the basis of their ability to form colonies on agar plates. Mean and s.d. of triplicate HeLa cultures and duplicate colony counts. siRNAs are further characterized in Supplementary Figure 2.

B-C) Hyperproliferation of *S.*Typhimurium, in a Lamp1-negative compartment, despite normal infectivity in cells depleted of galectin-8. HeLa cells transfected with the indicated siRNAs were infected with *S.*Typhimurium. B) At the indicated time points cells were scored according to the number of bacteria they contained. C) Cells were stained with an antibody against Lamp1. Lamp1-positive and negative bacteria were counted by microscopy at 6h post infection. Cells were scored according to the number of bacteria they contained.

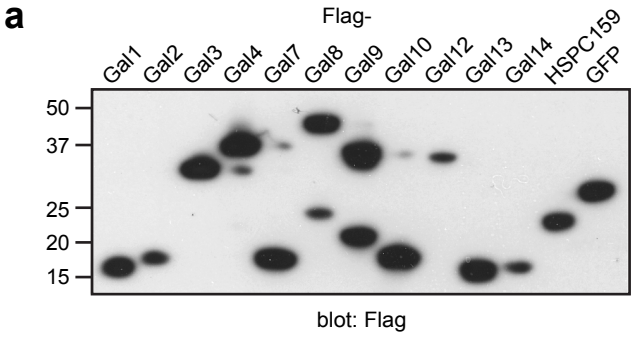
Mean and s.d. of duplicated coverslips, >200 bacteria were counted per coverslip.

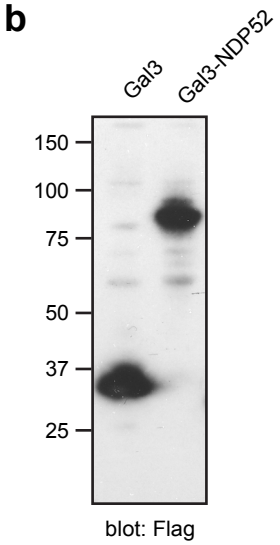

a
Salmonella (fold replication)
50
25
0
siControl
siControl+siGal8#38
siGal8#38 (doubled)
siGal3#669+siGal9#809
2
4
6
8
time p.i. (h)
b
100
50
0
Cells (%)
siControl
siGal8
bacteria per cell
1-10
11-20
>20
2h
4h
6h
c
70
35
0
Salmonella (%)
Lamp1+ve
Lamp1-ve
siControl
siGal8
1-10
11-20
>20
bacteria per cell

Supplementary Figure 4. Characterization of proteins.

A) Lysates of 293ET cells expressing the indicated Flag-tagged galectins, used in Fig.2a, were probed with anti-Flag antibody.

B) Lysates from HeLa cells expressing the indicated Flag-tagged galectin-3 variants, used in Fig.3g, were probed with anti-Flag antibody.


a
Flag-
Gal1
Gal2
Gal3
Gal4
Gal7
Gal8
Gal9
Gal10
Gal12
Gal13
Gal14
HSPC159
GFP
50
37
25
20
15
blot: Flag

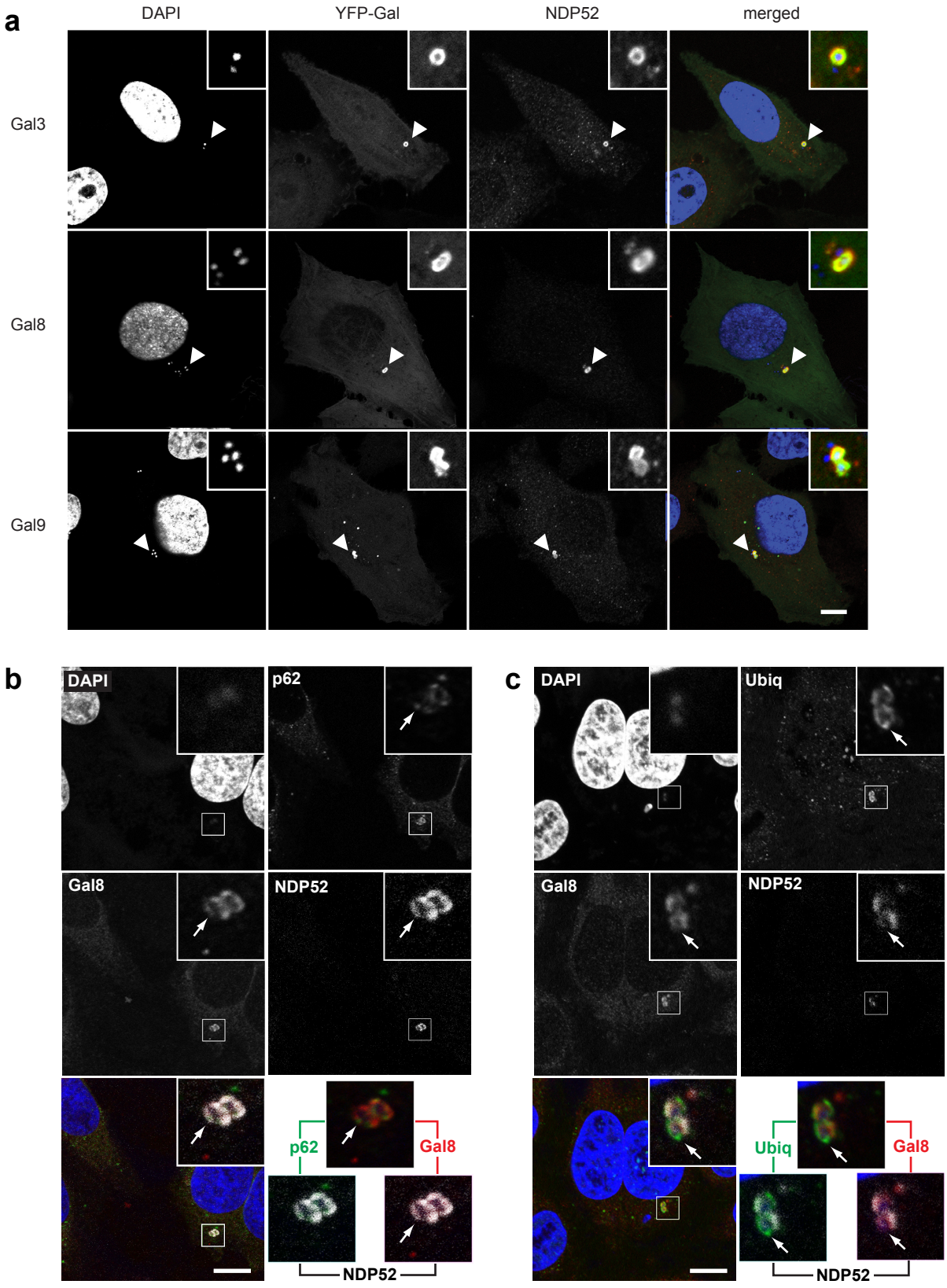

b
Gal3
Gal3-NDP52
150
100
75
50
37
25
blot: Flag

Supplementary Figure 5. Galectin-8 and NDP52 form microdomains on bacteria distinct from p62 and ubiquitin.

A) Galectins and NDP52 co-localize. Confocal micrographs of HeLa cells stably expressing YFP fused to the indicated galectins, infected with *S*.Typhimurium and stained with NDP52 antiserum 1h after infection.

B-C) Bacterial coats comprise distinct microdomains. Confocal micrographs of HeLa cells infected with *S*.Typhimurium and stained at 1h post infection with DAPI and antibodies against (B) galectin-8, p62, and NDP52 and (C) galectin-8, ubiquitin, and NDP52. White arrows in insets indicate the position of (B) p62 and (C) ubiquitin clusters.

Scale bar 10 μm.


a
DAPI
YFP-Gal
NDP52
merged
Gal3
Gal8
Gal9
b
DAPI
p62
Gal8
NDP52
p62
Gal8
NDP52
c
DAPI
Ubiq
Gal8
NDP52
Ubiq
Gal8
NDP52

Supplementary Figure 6. Defining the sites of interaction in NDP52 and galectin-8.

A) Domain structure of galectin-8 and NDP52.

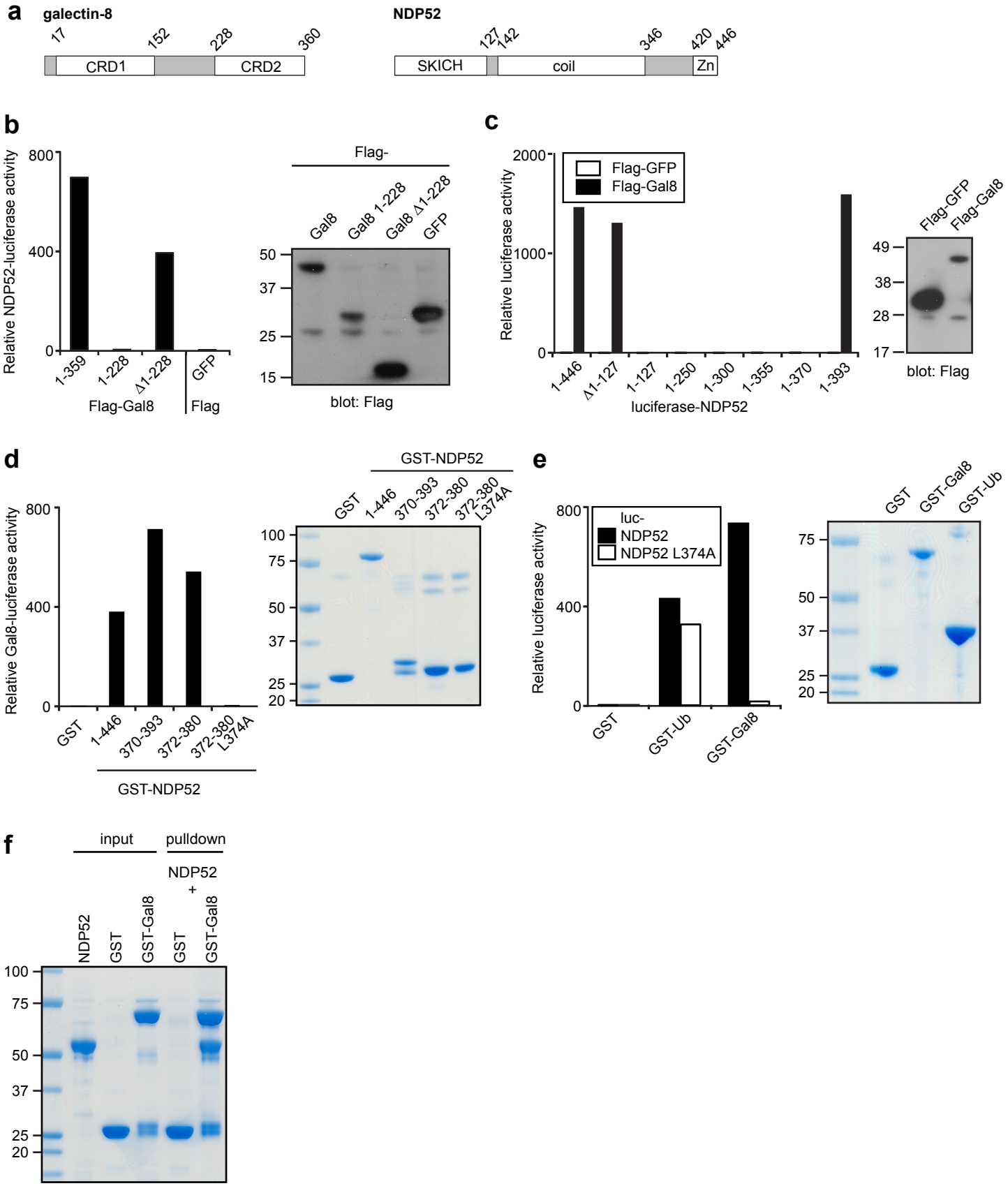
B-E) LUMIER binding assay: Normalized ratio between luciferase activity bound to beads and present in lysates.

B) Left panel: lysates from 293ET cells expressing the indicated Flag-tagged proteins and NDP52 fused to luciferase were incubated with anti-Flag beads. Right panel: lysates from 293ET cells expressing the indicated Flag-tagged proteins were probed with anti-Flag antibody.

C) Left panel: lysates from 293ET cells expressing Flag-tagged GFP or Gal8 and the indicated NDP52 variants fused to luciferase were incubated with anti-Flag beads. Right panel: lysates from 293ET cells expressing the indicated Flag-tagged proteins were probed with anti-Flag antibody.

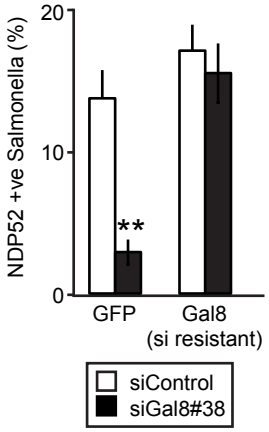
D-E) Left panels: the indicated purified GST fusion proteins coupled to beads were incubated (D) with lysates of *E. coli* expressing galectin-8 fused to luciferase or (E) with lysates of 293ET cells expressing the indicated NDP52 variants fused to luciferase. Right panels: proteins purified from bacteria expressing the indicated proteins were stained with Coomassie Blue.

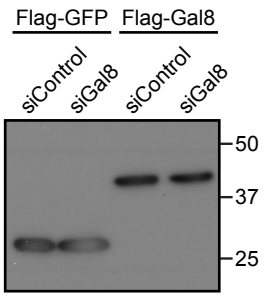
F) The indicated purified GST proteins bound to beads were incubated with purified NDP52 (input). Pulldown: proteins eluted from beads.


a
galectin-8
17
152
228
360
CRD1
CRD2
NDP52
127
142
346
420
446
SKICH
coil
Zn
b
Relative NDP52-luciferase activity
800
400
0
1-359
1-228
Δ1-228
GFP
Flag-Gal8
Flag
Flag-
Gal8
Gal8 1-228
Gal8 Δ1-228
GFP
50
37
25
15
blot: Flag
c
Relative luciferase activity
2000
1000
0
Flag-GFP
Flag-Gal8
1-446
Δ1-127
1-127
1-250
1-300
1-355
1-370
1-393
luciferase-NDP52
Flag-GFP
Flag-Gal8
49
38
28
17
blot: Flag
d
Relative Gal8-luciferase activity
800
400
0
GST
1-446
370-393
372-380
372-380 L374A
GST-NDP52
GST-NDP52
100
75
50
37
25
20
e
Relative luciferase activity
800
400
0
luc-
NDP52
NDP52 L374A
GST
GST-Ub
GST-Gal8
GST
GST-Gal8
GST-Ub
75
50
37
25
20
f
input
pulldown
NDP52
+
NDP52
GST
GST-Gal8
GST
GST-Gal8
100
75
50
37
25
20

Supplementary Figure 7. Complementation of galectin-8 knockdown restores NDP52 recruitment to *S.*Typhimurium.

Left panel: Colocalization of NDP52, visualized by an antiserum, with *S.*Typhimurium at 1h p.i. in HeLa cells treated with the indicated siRNAs and stably expressing FLAG-tagged GFP or siRNA-resistant FLAG-tagged galectin-8. Right panel: lysates were probed with a FLAG antibody. Mean and s.d. of duplicated HeLa cultures, n>200 bacteria per coverslip.

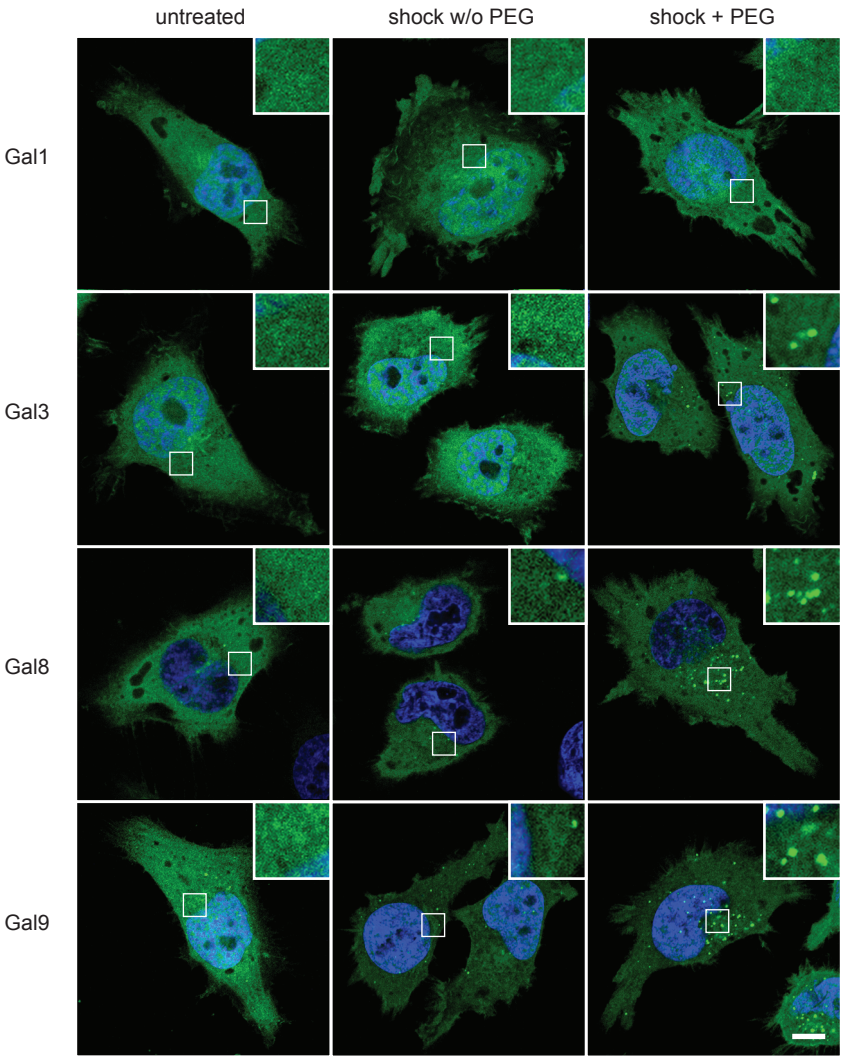

20
10
0
NDP52 +ve Salmonella (%)
**
GFP
Gal8
(si resistant)
siControl
siGal8#38


Flag-GFP
Flag-Gal8
siControl
siGal8
siControl
siGal8
50
37
25

Supplementary Figure 8. Galectins sense sterile damage to endosomes.

Confocal images of HeLa cells expressing the indicated YFP-tagged galectins. Cells were left untreated or were exposed to hypertonic conditions, with or without PEG as indicated, followed by hypotonic shock. (This figure is an extended version of Fig. 3f. Identical images for Galectin 1 and 8 are therefore shown in both figures.)

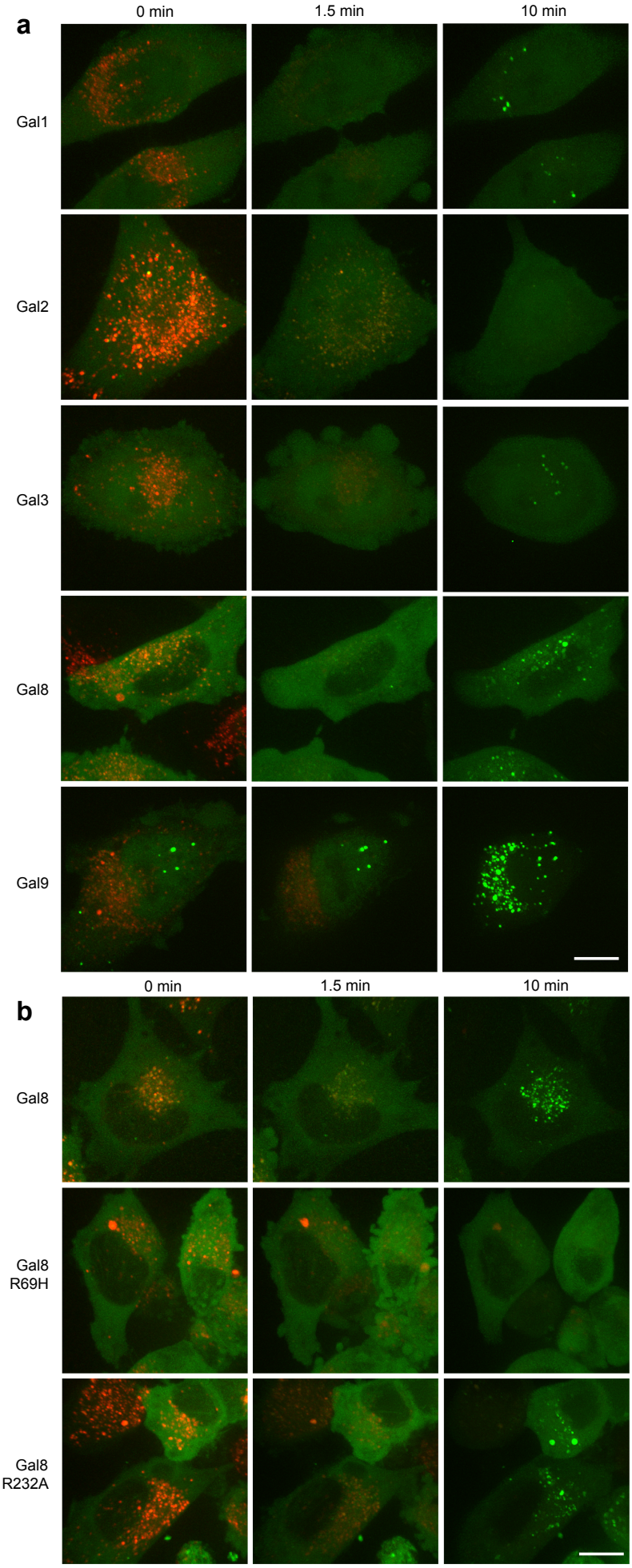
Scale bar 10μm.


untreated
shock w/o PEG
shock + PEG
Gal1
Gal3
Gal8
Gal9

Supplementary Figure 9. Galectins sense sterile damage to lysosomes.

A-B) Maximum intensity reconstruction of confocal Z-stacks of HeLa cells expressing the indicated YFP-tagged galectins. Cells stained with LysoTracker Red and treated with 333μM GPN were imaged at the indicated time points.

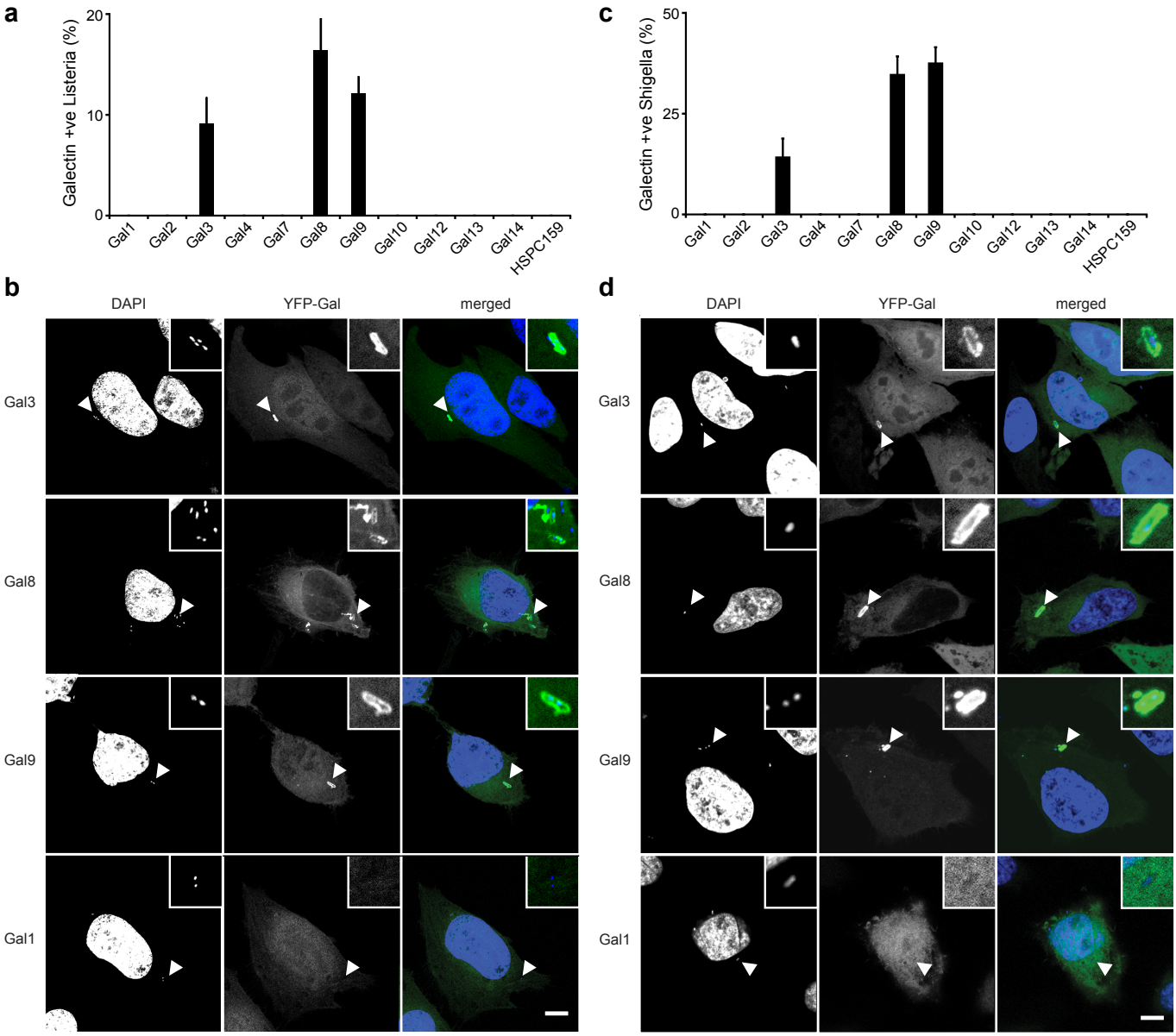
Scale bar 10μm.


a
0 min
1.5 min
10 min
Gal1
Gal2
Gal3
Gal8
Gal9
b
0 min
1.5 min
10 min
Gal8
Gal8
R69H
Gal8
R232A

Supplementary Figure 10. Galectins sense infection by *Listeria monocytogenes* and *Shigella flexneri.*

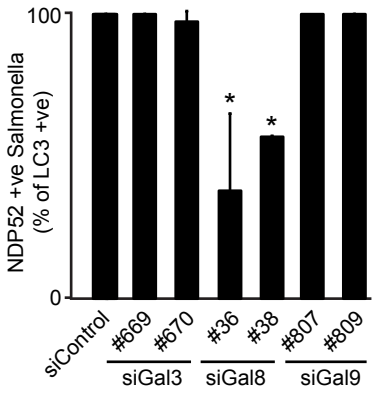
A-D) Analysis of HeLa cells stably expressing YFP fused to the indicated galectins and infected with (A-B) *Listeria monocytogenes* or (C-D) *Shigella flexneri* for 1h. A, C) Percentage of bacteria coated by the indicated galectins. YFP-positive bacteria were counted by microscopy. Mean and s.d. of duplicate HeLa cultures, n>200 bacteria per coverslip. B, D) Confocal micrographs. Arrowheads, bacteria shown in insets.

Scale bar 10μm.


a
Galectin +ve Listeria (%)
20
10
0
Gal1
Gal2
Gal3
Gal4
Gal7
Gal8
Gal9
Gal10
Gal12
Gal13
Gal14
HSPC159
c
Galectin +ve Shigella (%)
50
25
0
Gal1
Gal2
Gal3
Gal4
Gal7
Gal8
Gal9
Gal10
Gal12
Gal13
Gal14
HSPC159
b
DAPI
YFP-Gal
merged
Gal3
Gal8
Gal9
Gal1
d
DAPI
YFP-Gal
merged
Gal3
Gal8
Gal9
Gal1

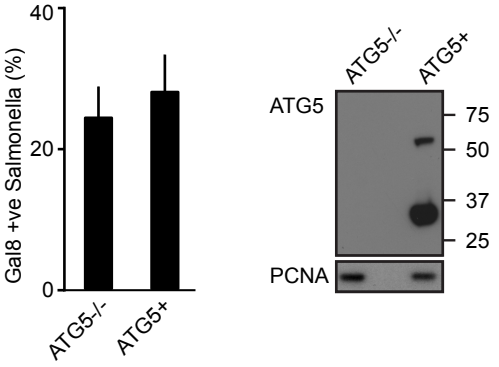
Supplementary Figure 11. Impaired co-localization of NDP52 and LC3 in cells depleted of galectin-8.

HeLa cells expressing GFP-LC3 were treated with the indicated siRNAs. Colcalization of NDP52, visualized by an antiserum, with GFP-LC3 on *S.*Typhimurium at 1h p.i. Mean and s.d. of duplicate HeLa culture, n>50 bacteria per coverslip


100
0
NDP52 +ve Salmonella
(% of LC3 +ve)
*
*
siControl
#669
#670
#36
#38
#807
#809
siGal3
siGal8
siGal9

Supplementary Figure 12. Autophagy is not required for the recruitment of galectin-8 to *S.*Typhimurium.

Left panel: Analysis of galectin-8 recruitment to *S.*Typhimurium in ATG5-deficient (ATG5-/-) or complemented (ATG5+) MEFs expressing YFP-tagged galectin-8. YFP-positive bacteria were counted by microscopy. Mean and s.d. of triplicate cultures, n>200 bacteria per coverslip. Right panel: lysates were probed with an ATG5 antibody.


40
20
0
Gal8 +ve Salmonella (%)
ATG5-/-
ATG5+
ATG5-/-
ATG5+
ATG5
PCNA
75
50
37
25