SUPPLEMENTAL DATA

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1. *H. pylori* binds to synthetic Le^b in a BabA-dependent manner.
H. pylori adhesion to immobilized Le^b on a microtiter plate was analyzed using an *in vitro* adhesion assay. The results represent the average of three separate experiments (each $n = 3$). Data are presented as the means ± SD (*error bars*).

Supplemental Figure S2. Establishment of cells stably expressing the ecotropic receptor.
MDCK or CHO cells stably expressing the murine ecotropic retrovirus receptor plasmid (MDCK/EcoR or CHO/EcoR) were infected with control (Control virus) or GFP retrovirus (GFP virus). After 5 days of infection, almost all of the cells infected with the GFP virus expressed GFP. *Scale bar*, 20 μm.

Supplemental Figure S3. Establishment of MEF cells expressing Le^b.
MEF cells stably expressing FUT1 and FUT3 (MEF/pIRES-FUT1-FUT3) were transduced with retroviruses expressing b3Gal-T5 (B3GALT5) or the control virus alone (Control virus). After 5 days of infection, the cells were fixed and stained with an anti-Le^b antibody (green) and rhodamine-labeled phalloidin (F-actin, red). *Scale bar*, 20 μm.

Supplemental Figure S4. Binding of *H. pylori* to Le^b-expressing NIH3T3 cells.
NIH3T3 cells were transduced with retroviruses expressing b3Gal-T5 (B3GALT5), FucT-I (FUT1), and FucT-III (FUT3), or the control virus alone (Control virus). After selecting with G418, the cells were infected with GFP-expressing ATCC 43579 *H. pylori* for 24 h at an MOI of 100. After fixation, the cells were stained with an anti-Le^b antibody (red). *Scale bar*, 20 μm.

Supplemental Figure S5. Binding of *H. pylori* to Le^b-expressing CHO cells.
CHO cells were either left untreated (−) or transduced with retroviruses expressing b3Gal-T5 (B3GALT5), FucT-I (FUT1), and FucT-III (FUT3), or the control virus alone (Control virus). After selecting with G418, the cells were infected with GFP-expressing ATCC 43579 *H. pylori* for 24 h at an MOI of 100. After fixation, the cells were stained with an anti-Le^b antibody (red). *Scale bar*, 20 μm.

Supplemental Figure S6. *H. pylori* binds to MDCK/Le^b but not MDCK/pIRES cells.
MDCK/Le^b or MDCK/pIRES cells were infected with GFP-expressing ATCC 43579 *H. pylori* for 24 h at an MOI of 100. After fixation, the cells were stained with an anti-Le^b antibody (red). *Scale bar*, 20 μm.

Supplemental Figure S7. *H. pylori* induces TFSS-dependent transcriptional activation in AGS cells in a BabA-independent manner.
AGS cells transfected with pNF-κB-luc (NF-κB), pNFAT (NFAT), pAP1-luc (AP-1) and pSRE-luc (SRE) vectors were infected with *H. pylori* for 5 h. The lysates were measured using the luciferase assay. The luciferase activity was calculated as the average fold induction compared to the control from at least three independent experiments. Data are presented as the means ± SD (*error bars*).

Supplemental Figure S8. *H. pylori* induces TFSS-dependent morphological changes in AGS cells in a BabA-independent manner.
AGS cells were infected with or without (–) the indicated *H. pylori* for 5 h at an MOI of 50. After fixation, the cells were stained with an anti-pY-CagA antibody (green), rhodamine-labeled phalloidin (red) and DAPI (DNA, blue). *Scale bar*, 20 μm.

Supplemental Table 1. *H. pylori* strains used in this study.

Parental strain	In text/figures as:	Relevant characteristics	Reference
ATCC 43504	WT		(23)
	Δ*babA*	Δ*babA::aph3*	This study
	Δ*virB7*	Δ*virB7::aph3*	(23)
NCTC 11637	WT		(20)
	Δ*babA*	Δ*babA::aph3*	This study
	Δ*virB7*	Δ*virB7::aph3*	(20)
J99	WT		(5)
	Δ*babA*	Δ*babA::aph3*	(5)
ATCC 43579	WT		(20)
	GFP	pHel3-GFP	This study and (20)

Supplemental Table 2. Oligonucleotides used in this study.

Purpose	ID	Sequence (5' to 3')
J99 BabA cloning forward	BabA-F	CGGTCGACATGAAAAAACACATCCTTTCATTAA CTTTAGGATCGC
J99 BabA cloning (C-terminal FLAG tag) reverse	BabA-R	CGGGGTACCTCACTTGTCATCGTCGTCCTTGTAG TCCGCGGATCCATAAGCGAACACGTAATTCAAA TACACGCT
FUT3 cloning forward	FUT3-F	CGAGATCTATGGATCCCCTGGGTGCAGC
FUT3 (C-terminal Myc tag) cloning reverse	FUT3-R	CCGCTCGAGTCACAGATCCTCTTCTGAGATGAG TTTTTGTTCGGTGAACCAAGCCGCTATGCTGC
FUT1 cloning forward	FUT1-F	CGGGATCCATGTGGCTCCGGAGCCATCG
FUT1 cloning (C-terminal FLAG tag) reverse	FUT1-R	CCGCTCGAGTCACTTGTCATCGTCGTCCTTGTAG TCAGGCTTAGCCAATGTCCAGAG
B3GALT5 cloning forward	B3GAL T5-F	CGGGATCCATGGCTTTCCCGAAGATGAG
B3GALT5 cloning (C-terminal HA tag) reverse	B3GAL T5-R	CCGCTCGAGTCAAGCATAATCAGGAACATCATA CGGATAACCCATGACAGGCGGACAATCTTC
GST cloning forward	GST-F	CCGATTAATATGTCCCCTATACTAGGTTATTG
GST cloning reverse with PreScission-recognition site	GST-R	GGAATTCCATATGGGGCCCCTGGAACAGA
H. pylori 16S ribosomal RNA real-time PCR forward	Hp 16S-F	CAACATGGCTGATTTGCG
H. pylori 16S ribosomal RNA real-time PCR reverse	Hp 16S-R	ACACCTCTCAGTTCGGA
Canis familiaris CCL5 real-time PCR forward	CCL5-F	TGCTTTGCCTACATTTCCGGCCGA
Canis familiaris CCL5 real-time PCR reverse	CCL5-R	AAAGACGACTGCTGGCATGGAGCA
Canis familiaris IL8 real-time PCR forward	IL8-F	TGGCTGTTGCTCTCTTGGCAGCTT
Canis familiaris IL8 real-time PCR reverse	IL8-R	TGGGATGGAAAGGTGTGGAGTGTGT
Canis familiaris CDX2 real-time PCR forward	CDX2-F	ACATCACCATCCGGAGGAAAGCTGA
Canis familiaris CDX2 real-time PCR reverse	CDX2-R	TGCTGCTGCTGCTGCAACTTCT
Canis familiaris MUC2 real-time PCR forward	MUC2-F	TGGAGGCGAGCATTGGTGTCATCA
Canis familiaris MUC2 real-time PCR reverse	MUC2-R	AACGCTGGTCAAAGTTGCCGCA
Canis familiaris GAPDH real-time PCR forward	GAPDH -F	ACCAGGGCTGCTTTTAACTCTGGCA
Canis familiaris GAPDH real-time PCR reverse	GAPDH -R	TGACTGTGCCGTGGAATTTGCCGT

Meriones unguiculatus CXCL1 real-time PCR forward	CXCL1-F	TCACCTTCAAGCTCTGGATGC
Meriones unguiculatus CXCL1 real-time PCR reverse	CXCL1-R	GCTCCAGTCGCCAACGAG
Meriones unguiculatus 18S ribosomal RNA real-time PCR forward	gerbil 18S-F	GGCTACCACATCCAAGGAAGG
Meriones unguiculatus 18S ribosomal RNA real-time PCR reverse	gerbil 18S-R	AGGGCCTCGAAAGAGTCCTG

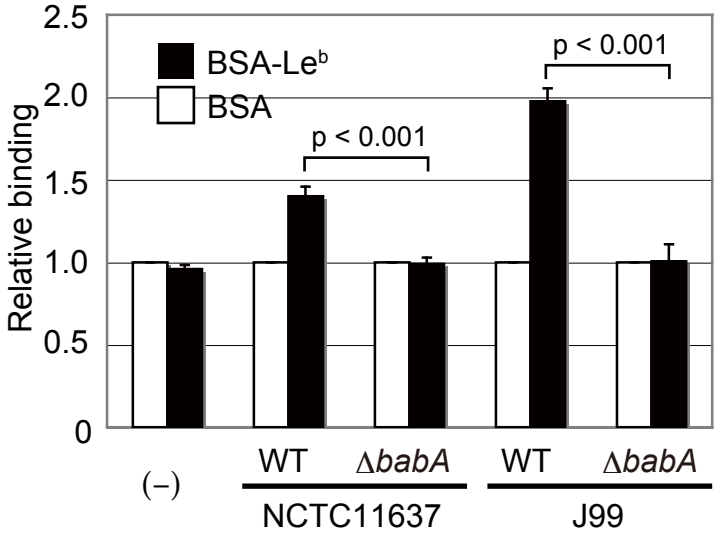
2.5
2.0
1.5
1.0
0.5
0
Relative binding
BSA-Le^b
BSA
p < 0.001
p < 0.001
(–)
WT
ΔbabA
WT
ΔbabA
NCTC11637
J99

Figure S1

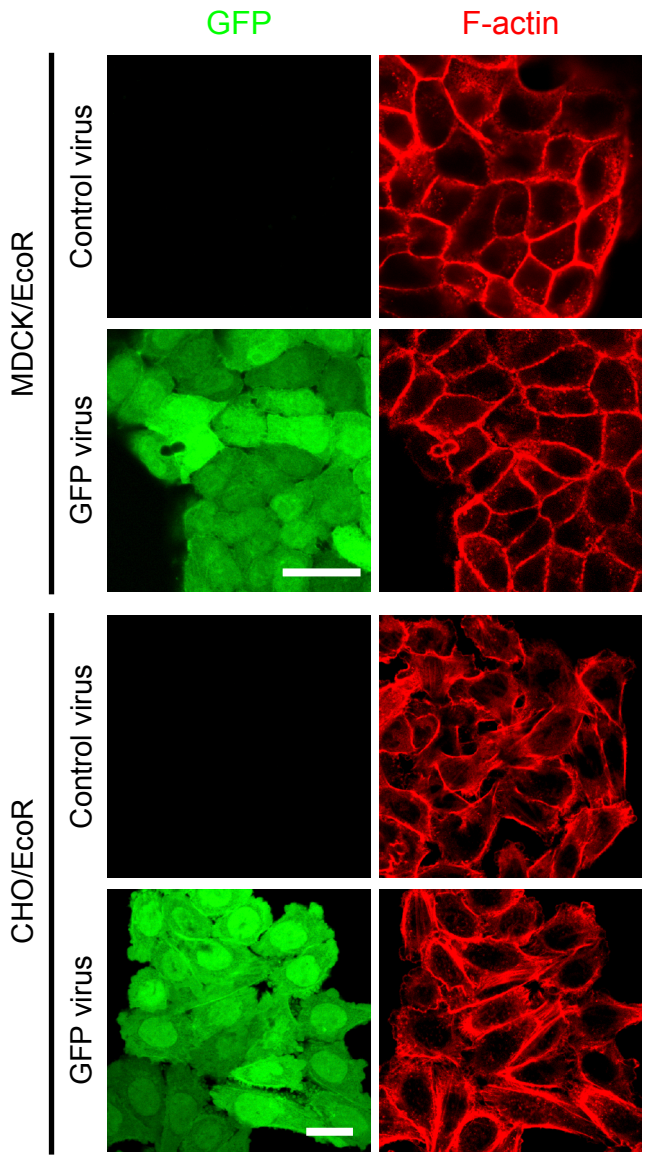
GFP
F-actin
MDCK/EcoR
Control virus
GFP virus
CHO/EcoR
Control virus
GFP virus

Figure S2

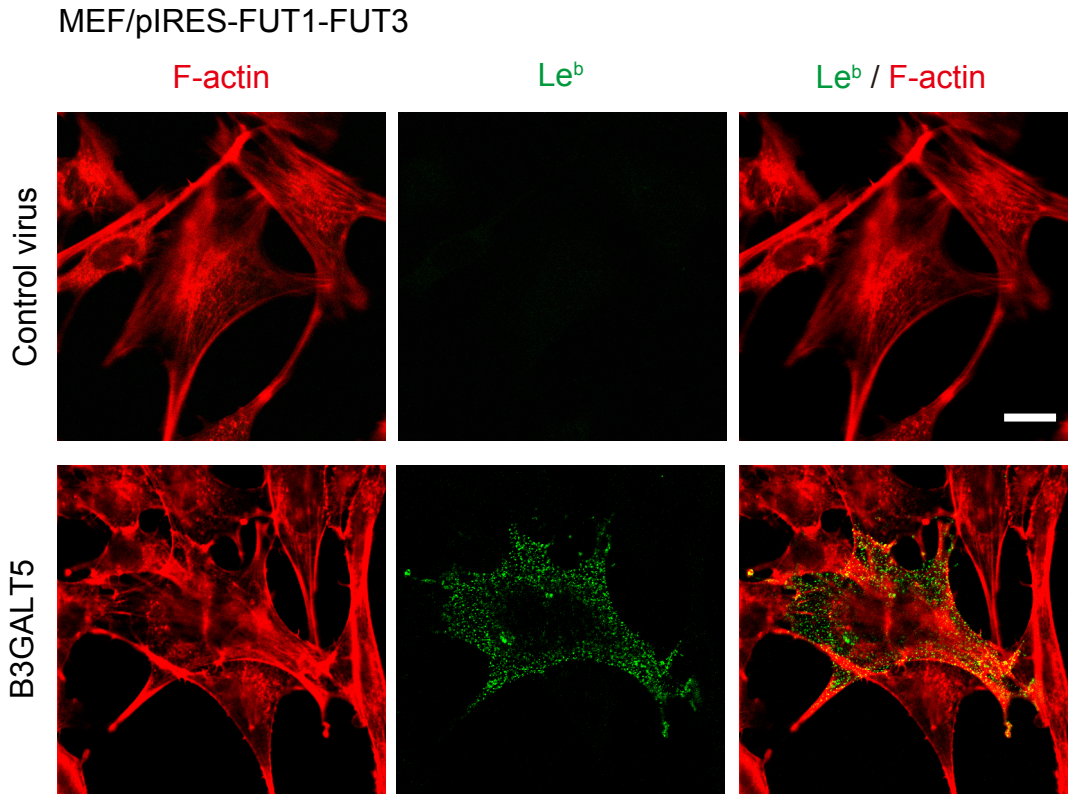
MEF/pIRES-FUT1-FUT3
F-actin
Leb
Leb / F-actin
Control virus
B3GALT5

Figure S3

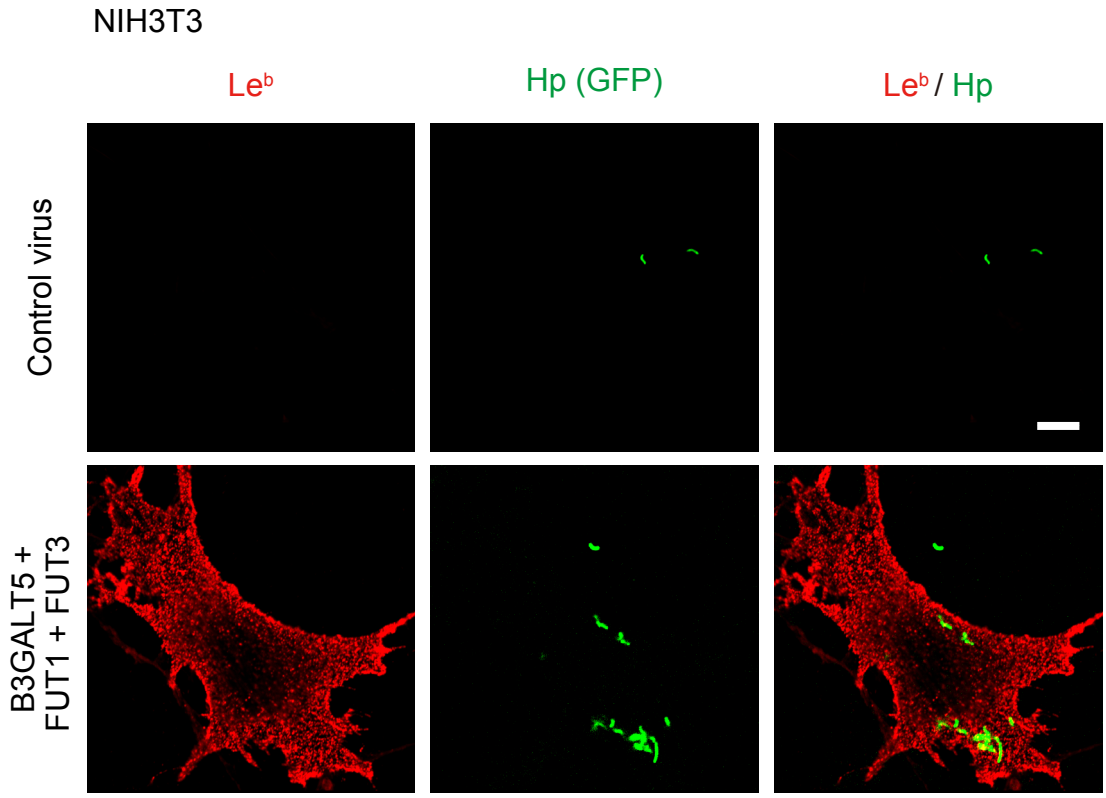

Figure S4

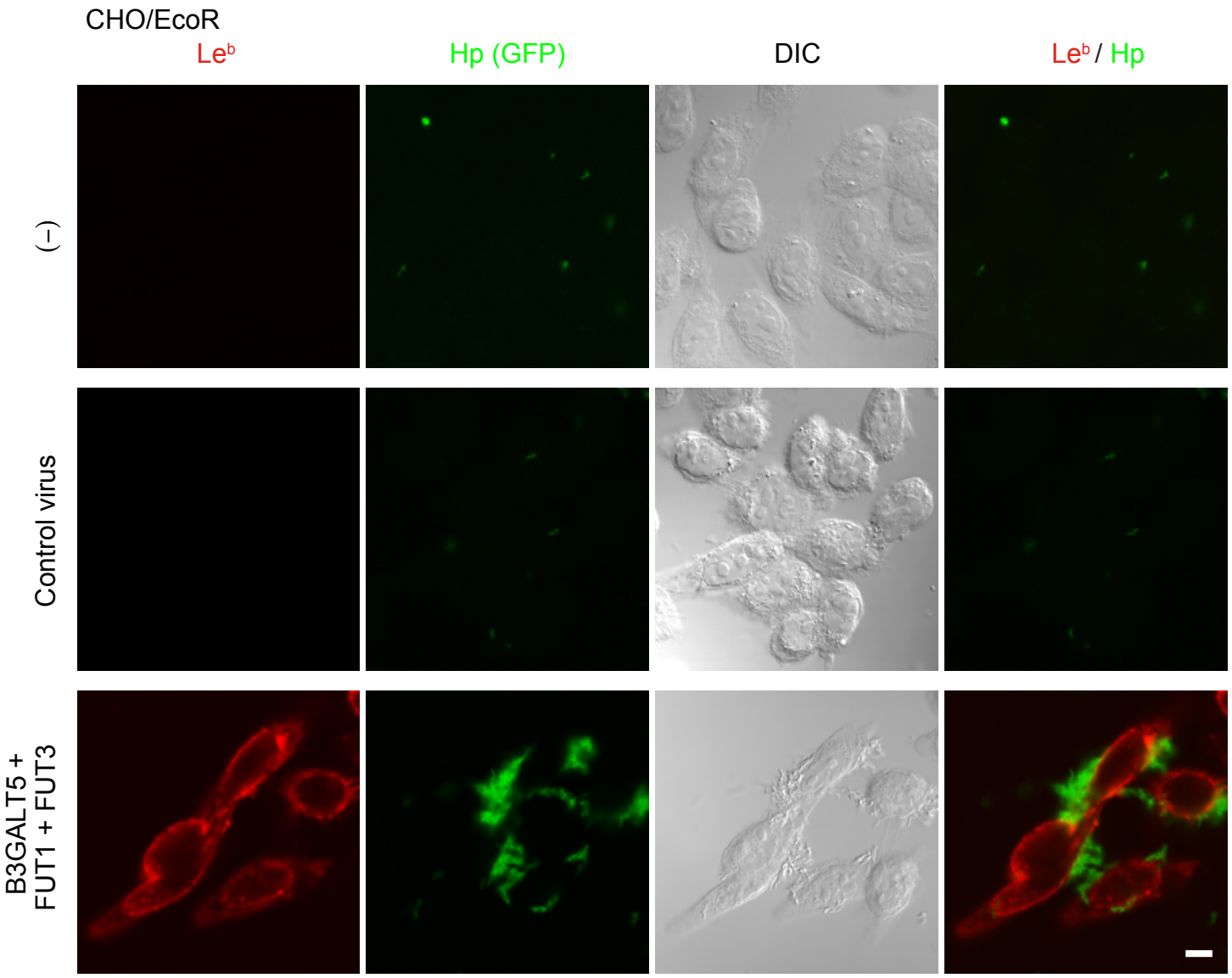
CHO/EcoR
Le^b
Hp (GFP)
DIC
Le^b / Hp
(–)
Control virus
B3GALT5 +
FUT1 + FUT3

Figure S5

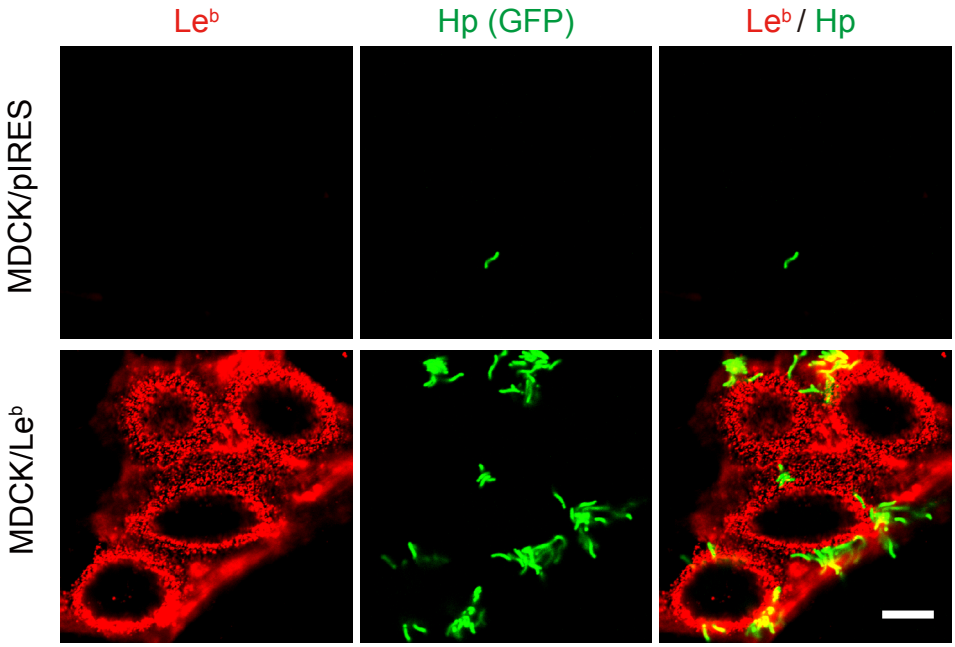

Figure S6

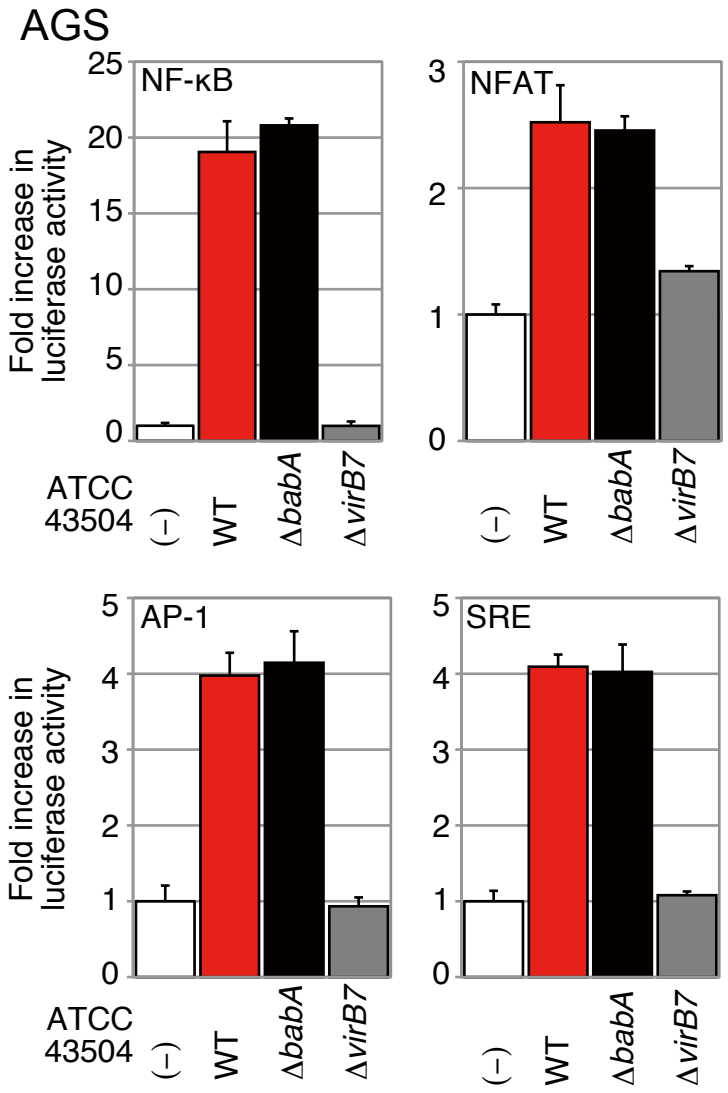
AGS
NF-κB
NFAT
AP-1
SRE
Fold increase in luciferase activity
25
20
15
10
5
0
3
2
1
0
5
4
3
2
1
0
ATCC 43504
(–)
WT
ΔbabA
ΔvirB7

Figure S7

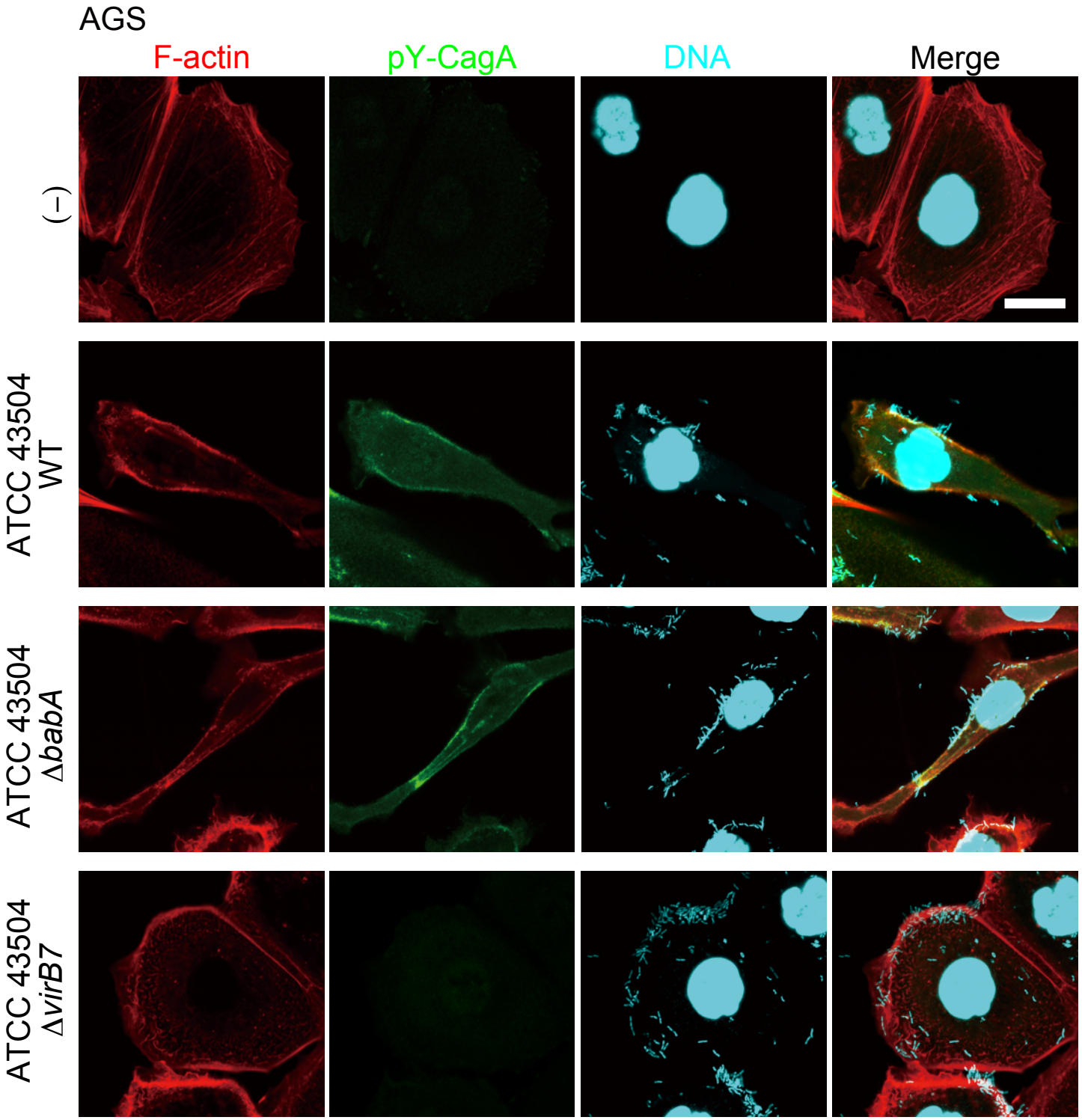
AGS
F-actin
pY-CagA
DNA
Merge
(–)
ATCC 43504
WT
ATCC 43504
ΔbabA
ATCC 43504
ΔvirB7

Figure S8