Supplementary information

Figure S1. Effect of AZM on NTHi infection of NCI H-292 human bronchoepithelial cells. (A) Cells were pre-treated with AZM 50 μg/ml. AZM was removed and infection was performed in AZM-free cell culture medium. Controls (CON): cells were treated with gentamicin but did not receive AZM. Mean numbers for NTHi375 entry into AZM pre-treated were significantly lower than those obtained for control cells ($p<0.0001$). NCI H-292 cells were infected with NTHi375 and AZM 50 μg/ml was added during cell incubation with gentamicin. Mean numbers for intracellular NTHi375 in AZM treated- were significantly lower than those obtained for control cells ($p<0.0001$). NCI H-292 cells were pre-treated with AZM 0.125 μg/ml. AZM was removed and infection was performed in AZM-free cell culture medium. Mean numbers for NTHi375 entry in AZM pre-treated were similar to those obtained for control cells. **(B)** Cells were pre-treated with AZM 50 μg/ml. AZM was removed and infection was performed in AZM-free cell culture medium. Controls (CON): cells were treated with gentamicin but did not receive AZM. Mean numbers for NTHi353 entry in AZM pre-treated were similar to those obtained for control cells. NCI H-292 cells were infected with NTHi353 and AZM 50 μg/ml was added during cell incubation with gentamicin. Mean numbers for intracellular NTHi353 in AZM treated- were significantly lower than those obtained for control cells ($p<0.005$). NCI H-292 cells were pre-treated with AZM 0.25 μg/ml. AZM was removed and infection was performed in AZM-free cell culture medium. Mean numbers for NTHi353 entry in AZM pre-treated were similar to those obtained for control cells.

Figure S2. Effect of the number of AZM prophylactic doses on NTHi375 bacterial counts in lungs. Mice were infected intranasally with ~10^8 bacteria/mouse. AZM (100 mg/kg/dose) was administered orally. Controls (CON): animals were administered

vehicle solution but did not receive AZM. Bacterial counts were determined at 12 h PI in lungs (log_{10} c.f.u./lung). NTHi375 counts were significantly ($p<0.01$) lower in mice treated with AZM prior to (1, 12 and 24 h) and after (at 6 h PI) infection than in control mice. Groups of animals receiving one (1, 12 or 24 h) or two (1 and 24 h) AZM doses prior to to infection did not reduce lung bacterial loads.

Table S1. Score of NTHi375 and NTHi353 co-localisation with EEA1 or LAMP1 during infection of A549 cells.

Strain	Endocytic marker	Time of incubation with Gm (min)	Control (no AZM)	AZM pre-treatment	Gm+AZM
NTHi375	EEA1	15	39±2	49±0.6	44.2±3.5
		60	47.6±8.1	43.5±4.8	43.4±2.7
	LAMP1	15	38.1±3.7	46.6±6.1	41.3±1
		60	42.3±11.1	47.75±2.64	41.18±0.9
NTHi353	EEA1	15	46.7±0.5	47.8±2.2	49.2±10.8
		60	49.2±6.2	47.03±4.1	54.95±6.7
	LAMP1	15	42.8±8.4	43.5±4.35	42.5±5.8
		60	50.6±8.4	52.8±5.4	54.6±6.6

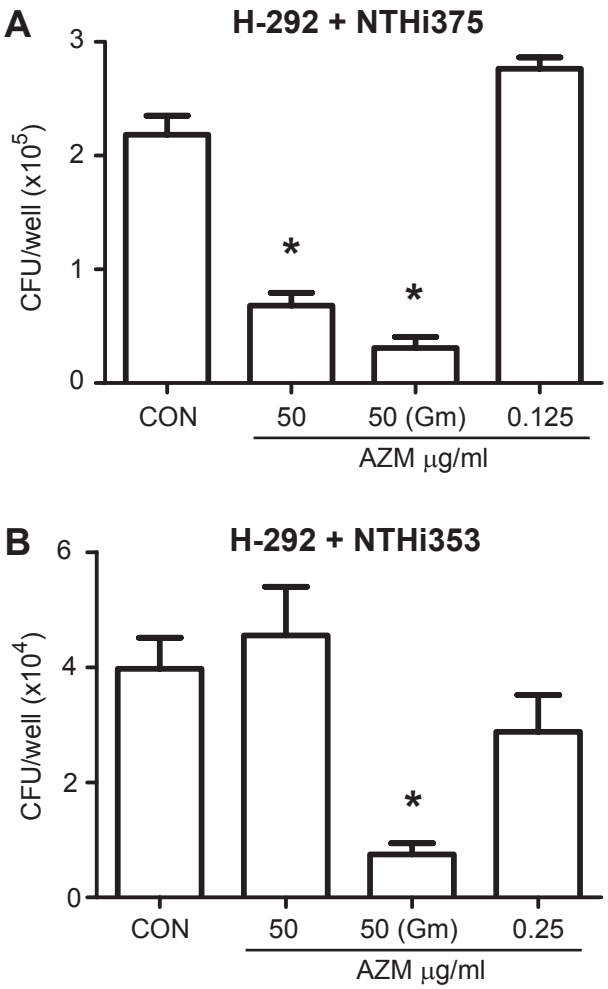
A
H-292 + NTHi375
CFU/well (x10^5)
3
2
1
0
*
*
CON
50
50 (Gm)
0.125
AZM μg/ml
B
H-292 + NTHi353
CFU/well (x10^4)
6
4
2
0
*
CON
50
50 (Gm)
0.25
AZM μg/ml

Figure S1

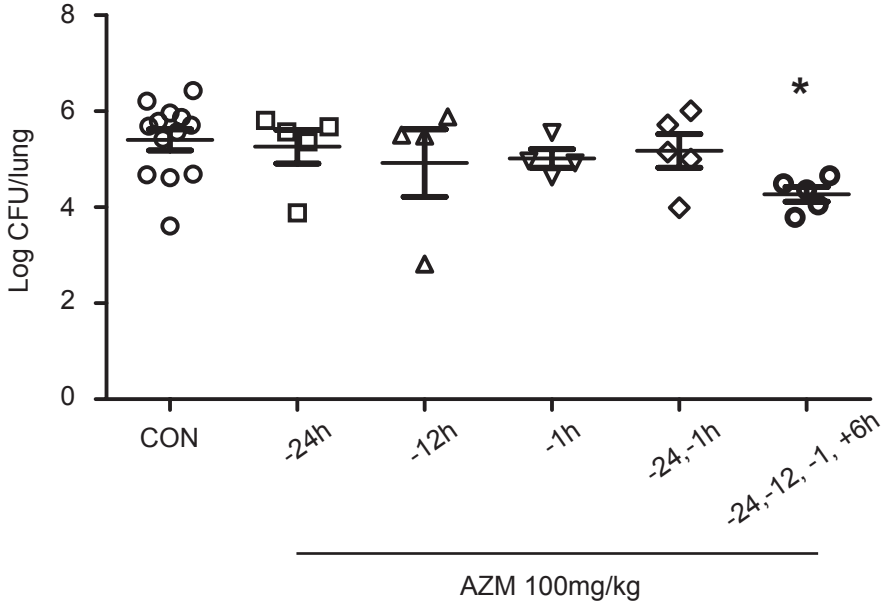

Figure S2