Supplementary Information

Table S1. Primers used in this study.

Primer	**Sequence (5´-3´)**
IL8-F	AGAGACAGCAGAGCACAC
IL8-R	AGTTCTTTAGCACTCCTTGG
AREG-F	TCCACTCGCTCTTCCAACAC
AREG-R	ATGGTTCACGCTTCCCAGAG
CXCL1-F	GCAGGGAATTCACCCCAAGA
CXCL1-R	CTTCAGGAACAGCCACCAGT
DUSP1-F	ACAAGGCAGACATCAGCTCC
DUSP1-R	TCGCCTCTGCTTCACAAACT
EGFR-F	GTGTGATCCAAGCTGTCCCA
EGFR-R	CTGTATTTGCCCTCGGGGTT
TLR2-F	GTACCTGTGGGGCTCATTGT
TLR2-R	CAAGACCCACACCATCCACA
SIRT1-F	TCGCCTTGCTGTAGACTTCC
SIRT1-R	TTTGGATTCCCGCAACCTGT
GAPDH-F	GAAGGTGAAGGTCGGAGTC
GAPDH-R	(5'-GAAGATGGTGATGGGATTTC

Table S2. siRNAs used in this study.

Gene	**siRNA reference**	**Sequence (5´-3´)**
EGFR	SI02660140 (a)	TACGAATATTAAACACTTCAA
	SI02660147 (b)	ATAGGTATTGGTGAATTTAAA
TLR2	SI00050015 (a)	CTGGGCAGTCTTGAACATTTA
	SI00050022 (b)	CAGGTAAAGTGGAAACGTTAA
SIRT1	SI00098434 (a)	CAAGCGATGTTTGATATTGAA
	SI00098441 (b)	CAGGATTATTGTATTTACGTT
	Control AllStars	UUCUUCGAACGUGUCACG

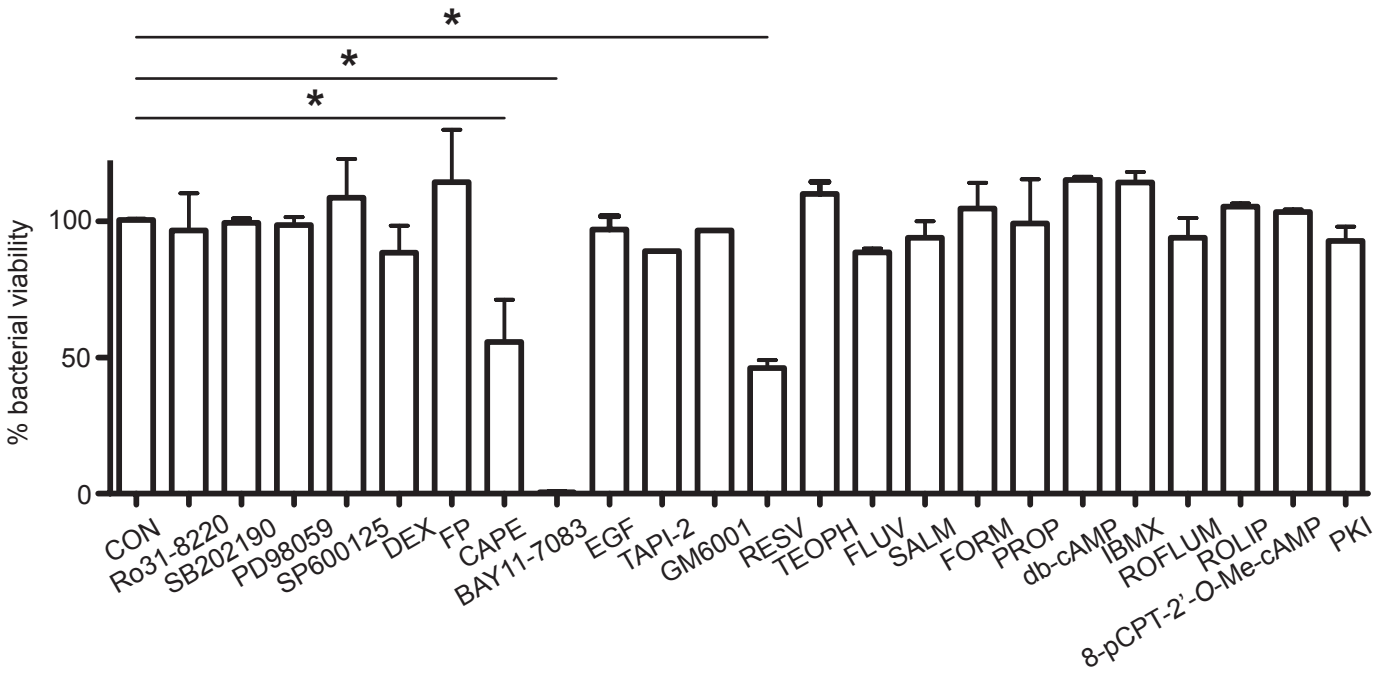

Figure S1. Effect of a panel of molecules on NTHi viability. Bacteria were collected from a freshly grown chocolate agar plate with 1 ml PBS, and adjusted to OD600=1; 100 µl of this suspension (~108 c.f.u.) were incubated in 1 ml EBSS medium for 2 h in the presence of Ro31-8220 1 µM, SB202190 30 µM, PD98059 50 µM, SP600125 50 µM, DEX 1 µM, FP 1 µM, CAPE 52.8 µM, BAY11-7093 5 µM, EGF 167 nM, TAPI-2 100 µM, GM6001 200 µM, RESV 20 µM, TEOPH 20 µM, FLUV 200 µM, SALM 1 µM, FORM 10 µM, PROP 20 µM, db-cAMP 1 mM, IBMX 1mM, ROFLUM 1 µM, ROLIP 10 µM, 8-pCPT-2′-O-Me-cAMP 100 µM, PKI 1 µM, or vehicle solution (control non-treated, CON). Bacterial viability was assessed by serial dilution and plating. Data are shown as per cent viability compared with CON non-treated bacteria considered to be 100%, given that vehicle solutions used in this study did not alter bacterial viability (data not shown). Data are the means of three independent experiments performed at least in triplicate. *$p<0.05$ compared to bacterial viability in the absence of treatment. CAPE, BAY11-7083 and resveratrol displayed a bactericidal effect.

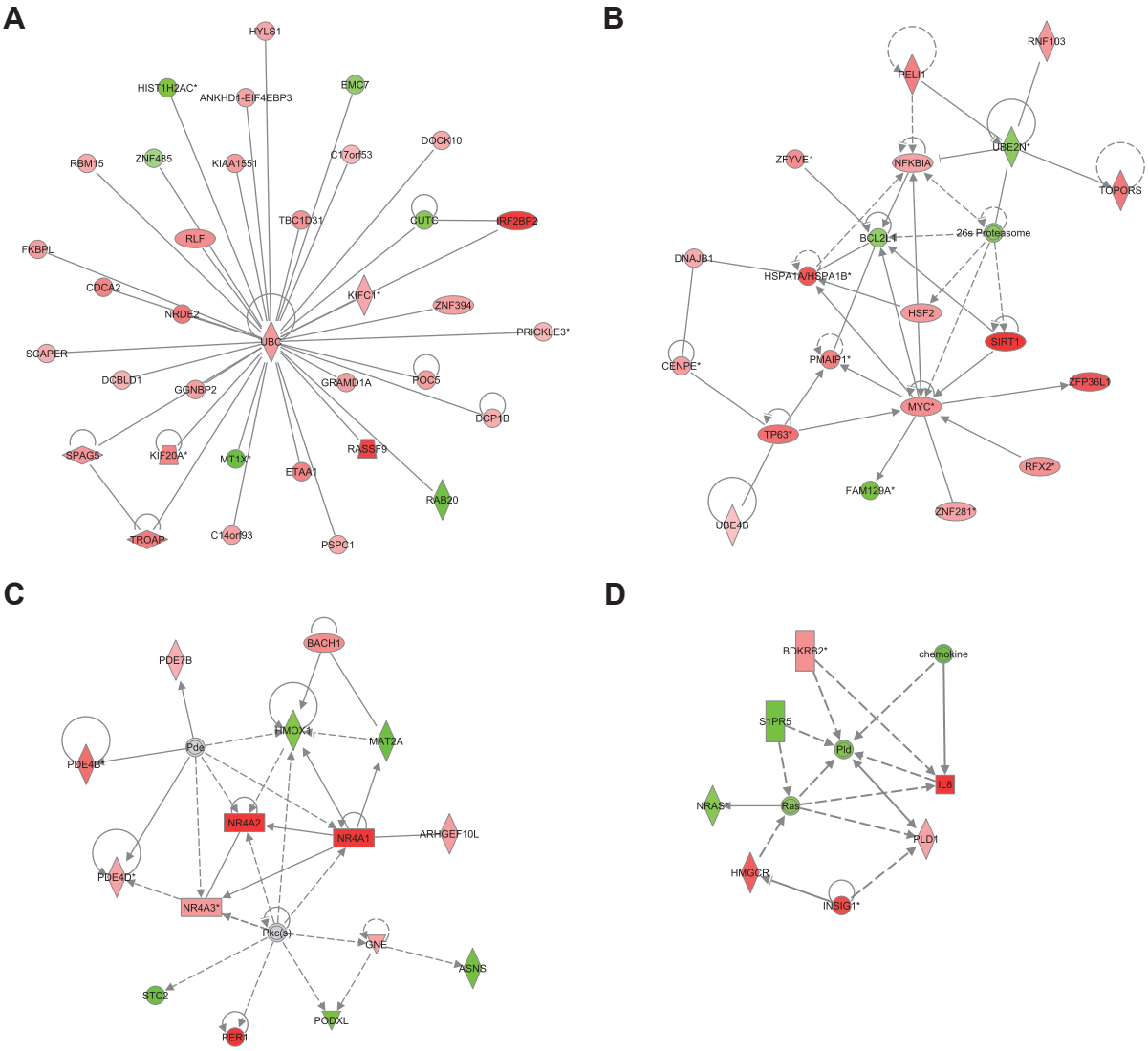

Figure S2. Networks of interactions between the selected genes obtained using IPA. (A) Network 1 (best scored network). (B) Network 3. (C) Network 8. (D) Network 21.

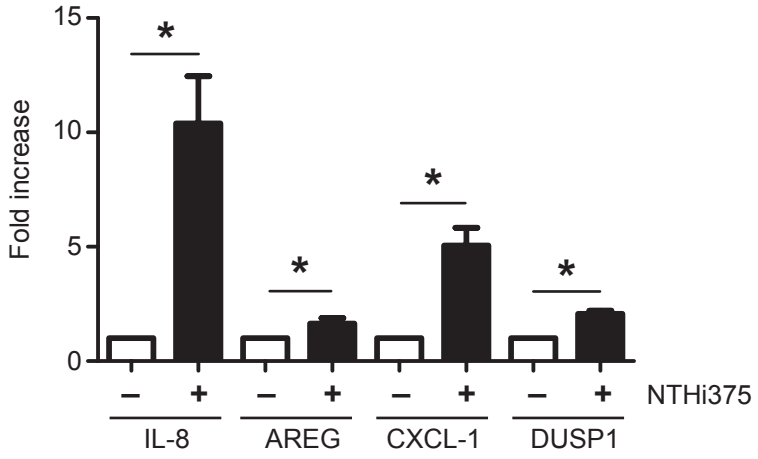

Figure S3. Relative comparison between gene expression in NTHi375-infected and control (CON) uninfected A549 cells. IL-8, AREG, CXCL-1 and DUSP1 mRNA levels, assessed by RT-qPCR in A549 control cells (CON) or cells infected with NTHi375 for 2 h (data are mean±SD; n=9). IL-8, AREG, CXCL-1 and DUSP1 mRNA levels were higher in NTHi375 infected- than in uninfected cells (IL-8, $p<0.05$; AREG, $p<0.05$; CXCL-1, $p<0.001$; DUSP1 $p<0.05$). Data are shown as gene expression in infected- compared to control uninfected cells.

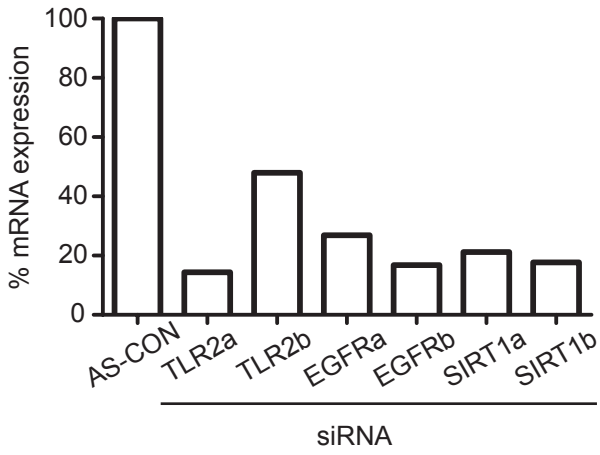

Figure S4. Validation of siRNA-mediated knockdown of TLR2, EGFR amd SIRT1. TLR2, EGFR and SIRT1 mRNA levels were measured by RT-qPCR on control (AS-CON) and siRNA treated cells. In all cases, a reduction of ~50-80% was observed in siRNA-treated compared to control (AS-CON) cells.

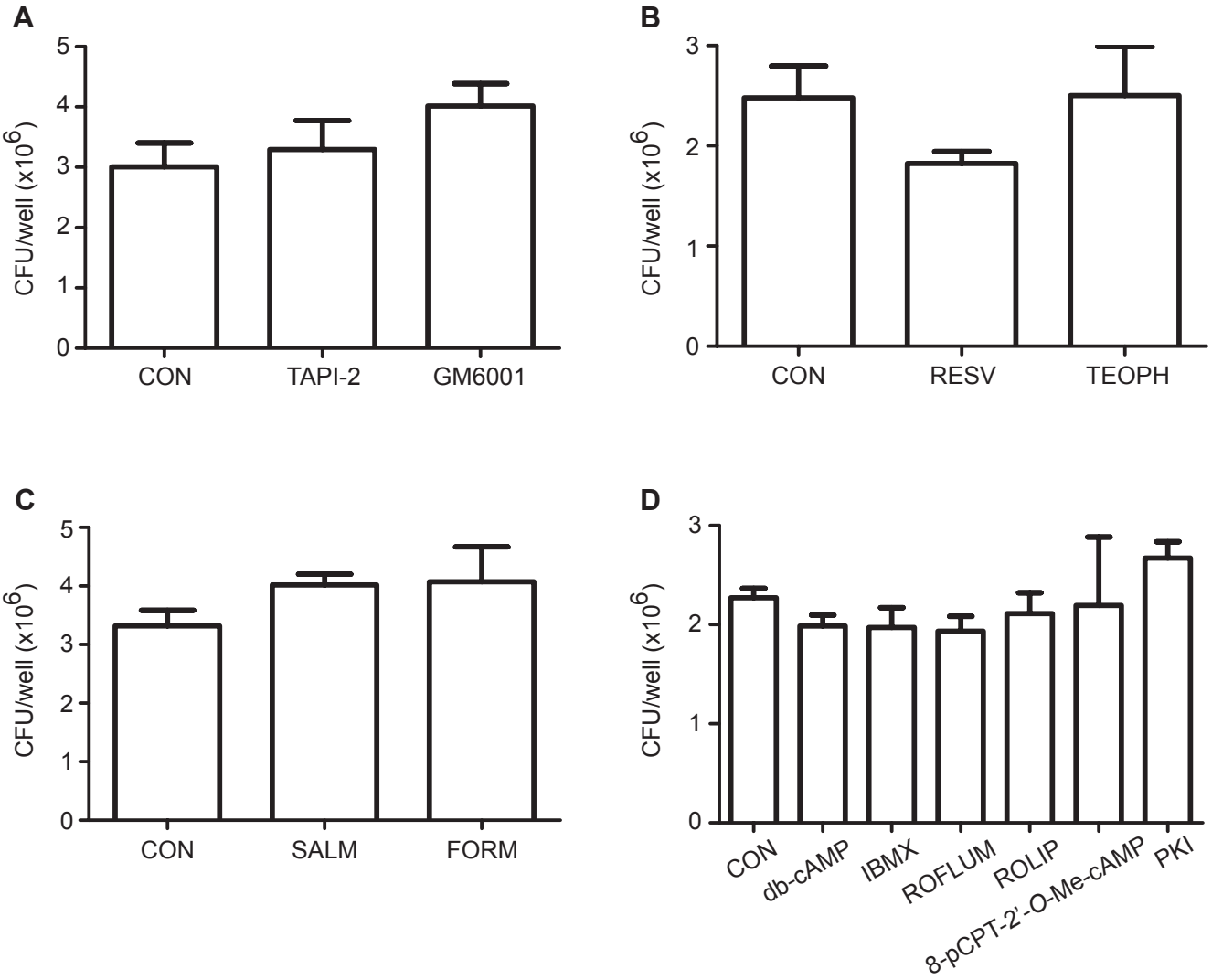

Figure S5. Effect of a panel of host-directed drugs on NTHi375 adhesion to A549 epithelial cells. Effect of cell treatment with TAPI-2 100 μM and GM6001 200 μM (A); RESV 20 μM and TEOPH 20 μM (B); SALM 1 μM and FORM 10 μM (C); db-cAMP 1 mM, IBMX 1mM, ROFLUM 1 μM, ROLIP 10 μM, 8-pCPT-2´-O-Me-cAMP 100 μM and PKI 1 μM (D) on NTHi375 adhesion to A549 cells. In all cases, comparable NTHi375 adhesion to treated- and control (CON) untreated cells was observed.