Supplemental Methods

NTHi infection & bacterial clearance assays *in vitro*

Macrophages and neutrophils were cultured in DMEM supplemented with 10% heat-inactivated fetal calf serum (FCS) (Sigma-Aldrich), 2mM L-glutamine, 0.1mM sodium pyruvate, and 20mM HEPES. *In vitro* macrophages were infected with MOI 100 NTHi for 8 h while neutrophils were infected with MOI 10 NTHi for 1 h. To measure extracellular bacteria the supernatants were first removed from the macrophage or neutrophil cultures. To measure intracellular bacteria load macrophages or neutrophils were incubated with gentamicin (400μg/mL) for 1 h at 37 ^{o}C to eliminate extracellular bacteria. Cells were then washed three times with PBS before being lysed with 0.25% saponin (Sigma-Aldrich) to release intracellular NTHi. Extracellular and intracellular bacteria were plated onto chocolate agar plates (Oxoid) and incubated overnight at 37 ^{o}C in an atmosphere of 5% CO_2. After 16 h, bacterial colonies were counted.

Measurement of uptake of heat-killed NTHi

Flow cytometry and immunofluorescence microscopy were used to measure the uptake of CFSE labelled heat-killed NTHi. NTHi was labelled with 100μM CFSE (Molecular Probes, before heat-killing) for 10 min at 37°C. Staining was quenched with FCS for 1 min, and bacteria washed, resuspended in PBS, and incubated with cells for 1 h. Cells were harvested for flow cytometry. Numbers of cells positive for CFSE and mean fluorescence intensity were measured using flow cytometry. To visualise the uptake of heat-killed NTHi, macrophages or neutrophils were initially adhered to coverslips. Cells were incubated with rhodamine and DAPI (Sigma-Aldrich) for 5 mins each to stain both cytoplasm and nucleus, respectively. Images were examined with an Olympus BX51 microscope with Olympus 40x objective and images captured using Olympus DP70 camera.

Inhibition of phagocytosis

Primary macrophages isolated from lungs were pretreated with ant-328 or Scr for 12 h before treatment with 2μg/mL Cytochalasin D (Sigma-Aldrich) or vehicle for 1 h. NTHi was labelled with 100μM CFSE (Molecular Probes, before heat-killing) for 10 mins at 37°C. Staining was quenched with FCS for 1 min, and bacteria washed, resuspended in PBS, and incubated with cells for 1 h. Cells were harvested for flow cytometry. Trypan blue (Life Technologies) were used to quench CFSE fluorescence from extracellular bacteria.

miRNA microarray analysis

Mice were challenged with either NTHi or PBS as control and were sacrificed at 24 h post-challenge. Total RNA was extracted from the airways using the Ambion mirVANA kit (Life Technologies) according to the manufacturer's protocol. RNA quality was determined using a bioanalyzer (Agilent). miRNA microarray analysis was performed with mouse miRNA microarray kit release 12.0 (Agilent) according to the manufacturer's protocol. Analysis of microarray data was conducted using Genespring GX software version 11.0.

43 **Isolation of mouse macrophages and neutrophils**

44 Lung tissue from naïve mice was forced through a 70μM cell strainer before being layered on 45 Histopaque 1083 (Sigma-Aldrich) and centrifuged (400 x g, 30min, 20°C). The interface 46 layer containing mononuclear cells was collected and allowed to adhere to a culture plate for 47 3 h at a concentration of 1 x 10^{6} cells before non-adherent cells were removed. Neutrophils 48 were extracted from the bone marrow of naïve mice using a 3 layer Percoll gradient (GE 49 Healthcare) and magnetic bead separation. Briefly, bone marrow cells were isolated from 50 femurs and tibias from both hind legs by flushing the bone with HBSS-EDTA solution. 51 Erythrocytes were lysed with lysis buffer. Cells were then layered on a three-layer Percoll 52 gradient of 78%, 69%, and 52% Percoll and centrifuged (1,500x*g*, 20 min, 20°C) at the 53 slowest deceleration. Cells from the 69%/78% interface were collected and neutrophils 54 further purified by negative immunomagnetic separation using labelling with anti-CD4, - 55 CD8, -CD11c, -CD49b, -CD117, -B220, and -F4/80 purified antibodies (Biolegend). 56 Labelled cells were depleted by magnetic separation by first incubating with BD IMAG 57 streptavidin particles plus-DM (BD Biosciences) according to the manufacturer's protocol. 58 The enriched fraction containing > 96% neutrophils was used.

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60 **Adoptive transfer**

61 Macrophages and neutrophils were pre-treated with antagomir miR-328 or scrambled control, 62 or were not exposed, and were harvested and labelled with 5μM of CFSE (Molecular Probes). 63 5 x 10^{5} CFSE labelled macrophages or neutrophils were transferred into the lungs of naïve 64 mice intratracheally in 40μL. 24 h after macrophage instillation and 2 h after neutrophil 65 instillation, mice were challenged with NTHi. Mice adoptively transferred with macrophages 66 were sacrifice 12 h post-inoculation while mice adoptively transferred with neutrophils were 67 sacrificed 6 h post-inoculation.

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69 **Cytokine analysis**

70 The concentrations of TNF-α and IL-6 were measured in the supernatants of BAL fluid and 71 lung homogenates, and *in vitro* cell culture supernatants using ELISA kits (eBioscience) 72 according to the manufacturer's instructions.

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74 **MAPK phosphorylation analysis**

75 Primary macrophages were treated with MOI 100 NTHi for 1 h. Cell were harvested to 76 analyse p38 and ERK activation by flow cytometry using BD Phosflow™ p38 MAPK and 77 ERK kit (BD Biosciences) according to manufacturer's protocol. Cell lysates were used to 78 measure JNK activation with Human/Mouse/Rat Phospho-JNK Pan specific DuoSet IC 79 ELISA kits (R&D Systems) according to manufacturer's protocol.

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81 **Isolation of human monocyte-derived macrohages and neutrophils**

82 Whole blood was obtained from healthy human adult volumteers with written informed 83 consent. PBMCs were isolated by Ficoll centrigugation (GE Healthcare) and cells were 84 adhered to cultures plates for 3 h at 5x10^{6} cells/ml after which non-adherent cells were gently

85 removed. Adherent cells were cultured in 50ng/ml recombinant human M-CSF (Peprotech) to
86 induce macrophage differentiation, and fresh media with M-CSF was replaced on days 3 and
87 6 of culture. Monocyte-derived macrophages were used on day 7. For neutrophil isolation the
88 remaining Ficoll layer was removed without disturbing the neutrophil/RBC layer. The thin
89 white cell layer of neutrophils above the RBC pellet was collected and resuspended in an
90 equal volume of HBSS and dextran/saline solution (5% dextran T500 in 0.9% NaCl at room
91 temperature), before being incubated in an upright position for 20 min at room temperature.
92 The layer of neutrophils above the sedimented RBC layer was aspirated and the remaining
93 RBCs lysed with lysis buffer. Cells were resuspended in culture media ready for assay.
94

95 **Synthesis of antagomir oligonucleotides**

96 The complementary antagomirs strands were generated using miRbase and were synthesised
97 by Sigma-Aldrich. miRNAs of interest were inhibited using the antagomirs outlined in table 1

98 **Table 1: Antagomirs design**

Antagomir	Sequence
Scrambled antagomir	5'mU.*.mC.*.mA.mC.mA.mA.mC.mC.mU.mC.mC.mU.mA.mG.mA.mA.mA.mG.mA.*.mG.*.mU.*.mA.*.3'-Chol
Antagomir-21	5'mU.*.mC.*.mA.mC.mA.mA.mC.mC.mU.mC.mC.mU.mA.mG.mA.mA.mA.mG.mA.*.mG.*.mU.*.mA.*.3'-Chol
Antagomir-21-3p	5'mU.*.mC.*.mA.mC.mA.mA.mC.mC.mU.mC.mC.mU.mA.mG.mA.mA.mA.mG.mA.*.mG.*.mU.*.mA.*.3'-Chol
Antagomir-146	5'mA.*.mA.*.mC.mC.mC.mA.mT.mG.mG.mA.mA.mT.mT.mC.mA.mG.mT.mT.mC.*.mT.*.mC.*.mA.*. 3'-Chol
Antagomir-376c	5'mU.*.mC.*.mA.mC.mA.mA.mC.mC.mU.mC.mC.mU.mA.mG.mA.mA.mA.mG.mA.*.mG.*.mU.*.mA.*.3'-Chol
Antagomir-223	5'mU.*.mG.*.mG.mG.mG.mU.mA.mU.mU.mU.mG.mA.mC.mA.mA.mA.mC.mU.mG.*.mA.*.mC.*.mA.*. 3'-Chol

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100 "m" represents 2'-OMe-modified phosphoramidites

101 "*" represents phosphorothioate linkages

102 “-Chol” was hydroxyprolinol-linked cholesterol to allow permeation of cell membranes

103

104 **mRNA quantitative polymerase chain reaction**

105 The sequence for the Muc5ac primers were: forward 5’-CGGCCGGAGAAAGTTGGTCCC -
106 3’, reverse 5’-GCACACCCGCCTGGTATGTCC-3’ (Sigma-Aldrich); CD14 primers were:
107 forward 5’-GGAAAGAAACTGAAGCCTTTCTCG-3’, reverse 5’-
108 AACAGCAACAAGCCAAGCACAC-3’ (Sigma-Aldrich); CD36_v2 primers were: forward
109 5’-GAGATGGCCTTACTTGGGATTGG-3’, reverse 5’-
110 GCCAGTGTATATGTAGGCTCATCCA-3’ (Sigma-Aldrich); CD11b_v2 primers were:
111 forward 5’-AAACTGCTCCTCAAAGCCATTGT-3’, reverse 5’-
112 GGTGACAATCATGTAGATGGCGTA-3’ (Sigma-Aldrich). The housekeeping gene
113 mHPRT was used as a control for the baseline level of cDNA expressed in all samples. The
114 sequence for the mHPRT primers were: forward 5’-AGGCCAGACTTTGTTGGATTTGAA-
115 3’, reverse 5’-CAACTTGCGCTCATCTTAGGCTTT-3’ (Sigma-Aldrich).

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117 **miRNA mimic transfection**

118 Primary macrophages were transfected with 50nM of miR-328 mimic or miR-Scr control
119 (Dharmacon) using Lipofectamine RNAiMAX reagent (Life Technologies) according to
120 manufacturer protocol.

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122 **Measuring lung function using forced oscillation technique (Flexivent)**

123 Lung function assessment was performed as reported previously [1]. Mice
124 were anaesthesised (i.p.) with 50 μL/10g mixture containing xylazine (2 mg/mL; Troy
125 laboratories, Smithfield, New South Wales, Australia) and ketamine (40 mg/mL;
126 Parnell, Alexandria, New South Wales, Australia). Tracheostomy was performed to
127 insert a cannula into the trachea. Mice were ventilated with a tidal volume of 8 mL/kg at
128 a frequency of 450 breaths/min and a positive end-expiratory pressure of 2 cm H2O.
129 FlexiVent perturbations were performed. Deep inflation, single compartment
130 (snapshot), pressure-volume loops with stepwise or increasing volumes (PVs-V), or
131 pressures (PVs-P), constant phase model (Primewave-8) perturbations were
132 performed. Primewave-8 perturbation was used for tissue elastance and snapshot
133 perturbations were used to measure compliance. Measurements were excluded if the
134 coefficient of determination was lower than 95%.

135

136 **Reference**

137 1. Li JJ, Wang W, Baines KJ, Bowden NA, Hansbro PM, et al. (2010) IL-27/IFN-gamma induce MyD88-
138 dependent steroid-resistant airway hyperresponsiveness by inhibiting glucocorticoid
139 signaling in macrophages. J Immunol 185: 4401-4409.

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