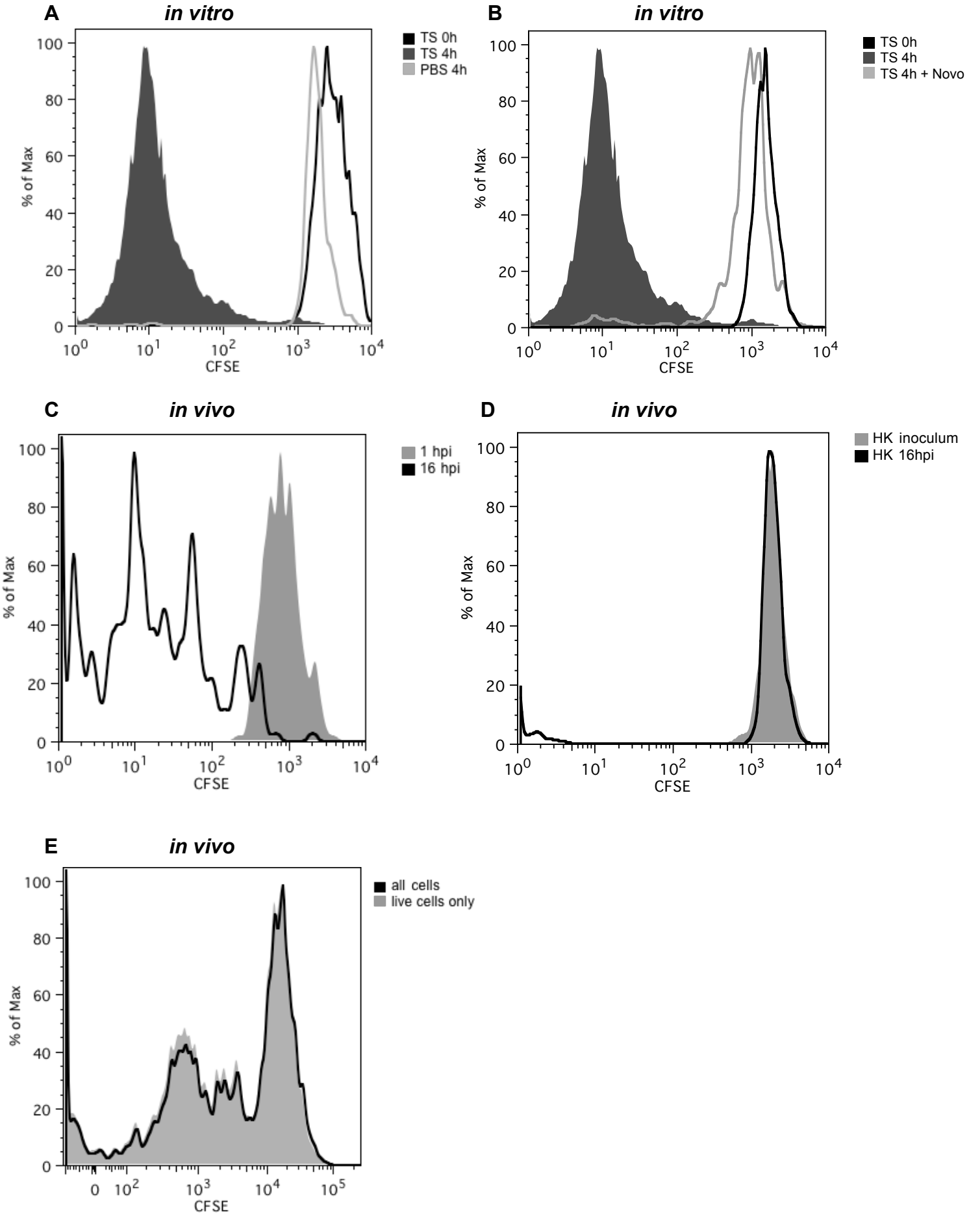
A
in vitro
TS 0h
TS 4h
PBS 4h
% of Max
CFSE
B
in vitro
TS 0h
TS 4h
TS 4h + Novo
% of Max
CFSE
C
in vivo
1 hpi
16 hpi
% of Max
CFSE
D
in vivo
HK inoculum
HK 16hpi
% of Max
CFSE
E
in vivo
all cells
live cells only
% of Max
CFSE

Figure S1: Bacterial replication can be measured *in vitro* and *in vivo* using CFSE, related to Figure 1

(A) CFSE-labeled pneumococci were grown *in vitro* in TS or PBS for 4 hrs. Flow cytometry was performed to measure the amount of CFSE fluorescence, and therefore the amount of replication, per cell. (B) CFSE-labeled pneumococci were incubated for 4 hrs in TS or TS containing bacteriostatic concentrations of the antibiotic novobiocin. (C) Mice were intranasally inoculated with CFSE-labeled pneumococci, and nasal lavages obtained 1 and 16 hrs later. Flow cytometry was performed to measure CFSE per cell at each timepoint. (D) Pneumococci were CFSE-labeled and then heat-killed (HK) to prevent subsequent growth. These heat-killed bacteria were inoculated into mice for 16 hrs, then nasal lavages obtained. Flow cytometry was performed and compared to the CFSE fluorescence of the heat-killed inoculum. (E) Viable CFSE-labeled pneumococci were inoculated into mice for 4 hrs, then nasal lavages obtained. Lavages were stained with propidium iodide and flow cytometry performed to compare CFSE fluorescence between all cells and live cells only.

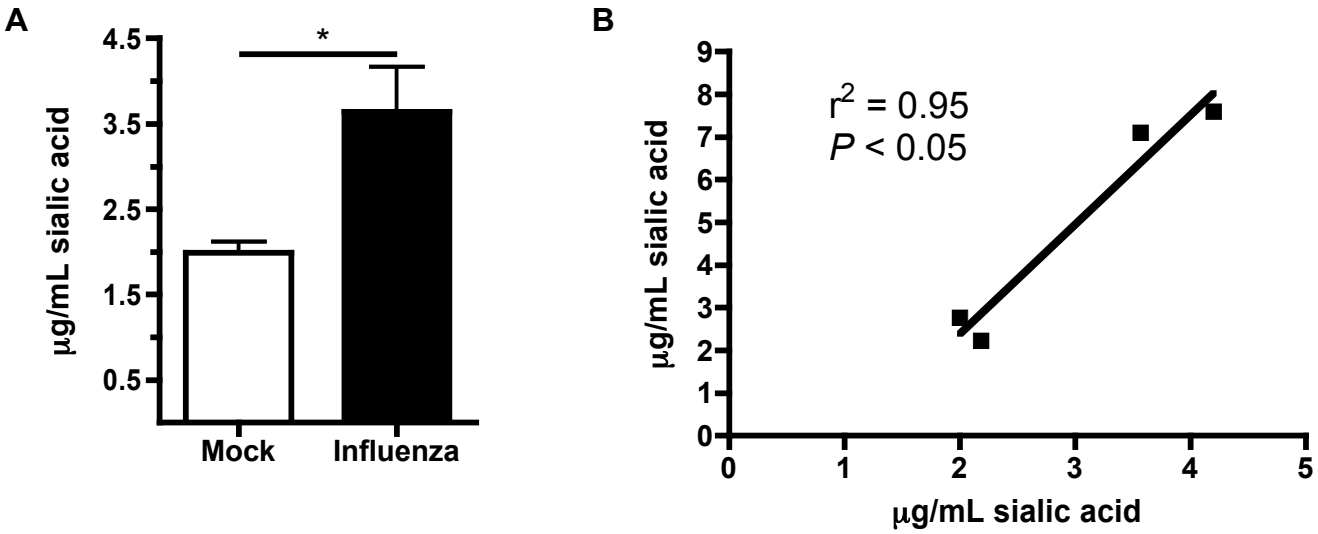
A
*
4.5
3.5
2.5
1.5
0.5
μg/mL sialic acid
Mock
Influenza
B
r2 = 0.95
P < 0.05
μg/mL sialic acid
μg/mL sialic acid
0
1
2
3
4
5
6
7
8
9

Figure S2: Validation of sialic acid measurements, related to Figure 2

(A) A subset of mock- and influenza-infected samples were analyzed for sialic acid content by DMB-derivatization and reverse-phase HPLC. (B) The same samples were analyzed by both thiobarbituric acid assay (y-axis) and DMB-HPLC (x-axis), and results from each method compared by linear regression. Nasal lavages from 3 mice were pooled into each sample analyzed, for a total of 6 mice per condition. Data are represented as mean +/- SD. * = $p < 0.05$

Supplemental Experimental Procedures:

CFSE Staining for Controls

CFSE-labeled pneumococci were incubated in TS or PBS (as a no-growth control) for 4 hrs, then fixed and stained for flow cytometry as described in Experimental Procedures. CFSE-labeled pneumococci were also incubated for 4 hrs in TS or TS containing bacteriostatic concentrations of the antibiotic novobiocin, a gyrase inhibitor that does not interfere with protein synthesis. To confirm the specificity *in vivo* of the CFSE assay for growing bacteria, pneumococci were CFSE-labeled and then heat-killed (HK) to prevent subsequent growth. The inoculum was plated to confirm no viable bacteria remained. These heat-killed bacteria were inoculated into mice for 16 hrs, then nasal lavages obtained. To verify that bacteria that appeared to be growing based on CFSE dilution were viable, CFSE-labeled pneumococci were inoculated into mice for 4 hrs, then nasal lavages obtained. Lavages were not fixed, but were stained with propidium

iodide and capsule type-specific antibody. Flow cytometry was performed, and the CFSE fluorescence per cell was compared between all cells and live cells only.

Sialic acid measurements

To validate the thiobarbituric acid assay, a subset of samples were also analyzed by DMB-derivatization and reverse-phase HPLC at the UCSD Glycotechnology Core. Briefly, free sialic acid was measured on half of each sample using a 10,000 MWCO spin filter. The other half of each sample was left unfiltered to measure total sialic acid. Each aliquot was hydrolyzed in 2 N acetic acid for 3 hrs at 80˚ C, followed by filtration through a 10,000 MWCO spin filter to remove protein. Samples were dried in a centrifugal evaporator and derivatized with DMB for 2.5 hrs at 55˚ C, followed by injection onto a Waters Acquity UPLC System. A Waters BEH C18 column (2.1 x 100 mm, 1.8µm particle size) was used, with a gradient of 7% Aqueous Methanol (Solvent A) and 7% Methanol in Acetonitrile (Solvent B) and initial conditions of 95%A and 5%B. The total run time was 14.5 min and the sialylated species were eluted from the column within the first 5 min. The amount of each sialic acid was determined by referencing to standards of known amounts.

qRT-PCR Primers

GAPDH-F 5'-AGG-TCG-GTG-TGA-ACG-GAT-TTG-3'; GAPDH-R 5'-TGT-AGA-CCA-TGT-AGT-TGA-GGT-CA-3'; Muc5ac-F 5'-CCA-TGC-AGA-GTC-CTC-AGA-ACA-A-3'; Muc5ac-R 5'-TTA-CTG-GAA-AGG-CCC-AAG-CA-3'; (Beisswenger et al., 2009) x31 NP-

F 5’-CAG-CCT-AAT-CAG-ACC-AAA-TG-3’; x31 NP-R 5’-TAC-CTG-CTT-CTC-AGT-TCA-AG-3’ (Hermesh et al., 2010).

Supplemental References

Beisswenger, C., Lysenko, E.S., and Weiser, J.N. (2009). Early bacterial colonization induces toll-like receptor-dependent transforming growth factor beta signaling in the epithelium. Infect. Immun. *77*, 2212–2220.

Hermesh, T., Moltedo, B., Moran, T.M., and López, C.B. (2010). Antiviral instruction of bone marrow leukocytes during respiratory viral infections. Cell Host Microbe *7*, 343–353.