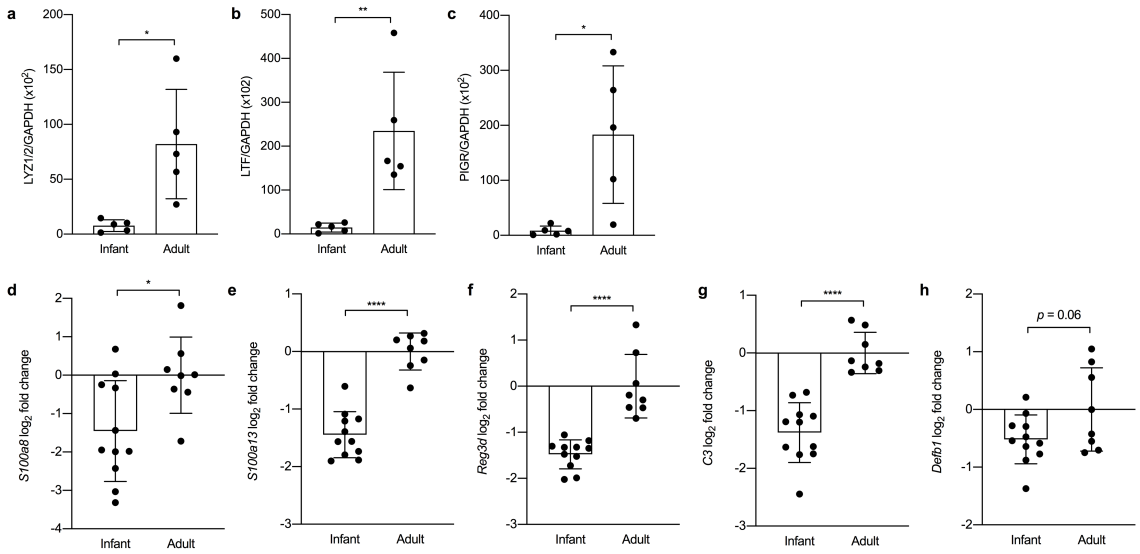
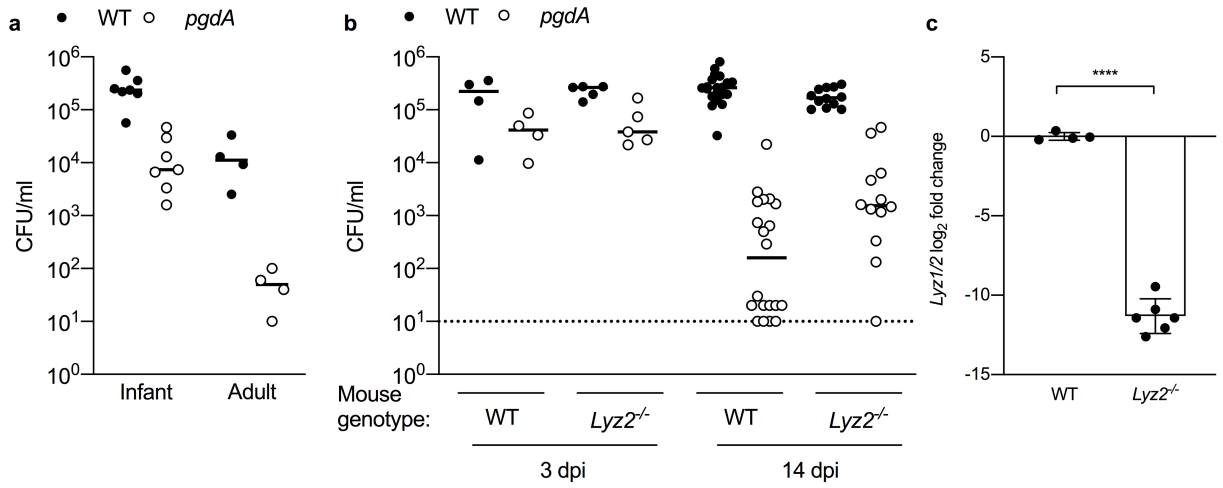
S Figure 1



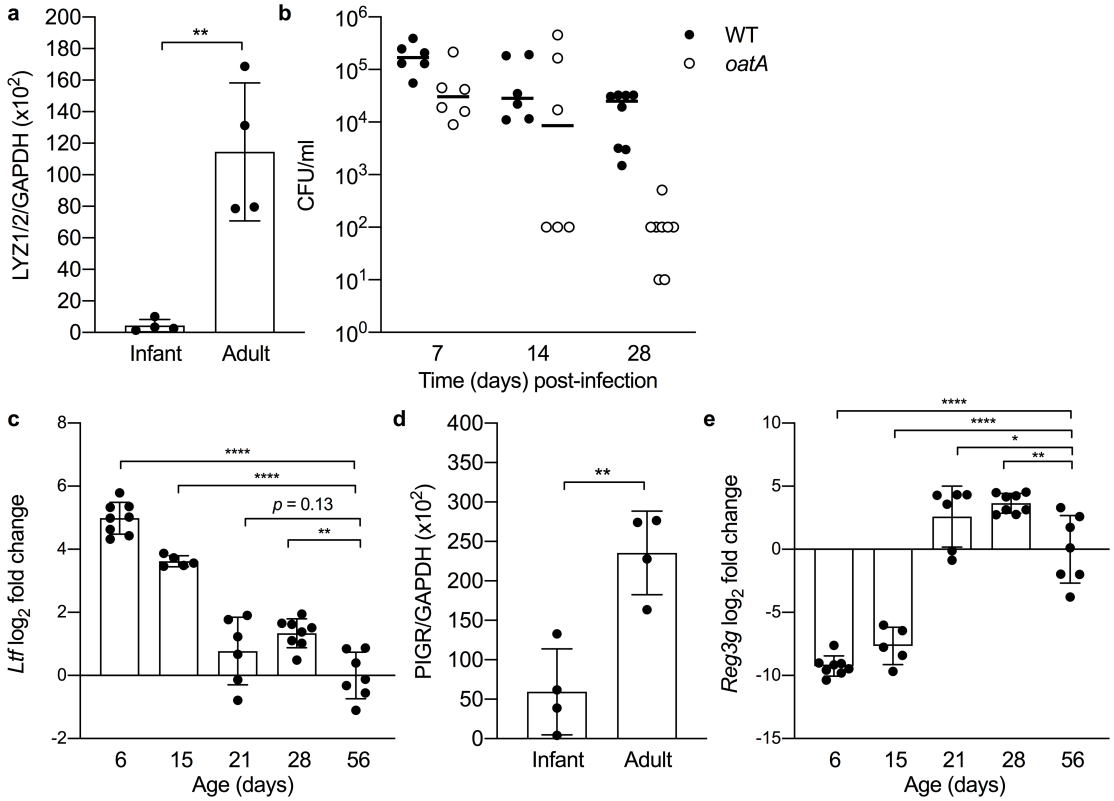
a-c, Densitometric quantification of (**a**) LYZ1/2 band intensity relative to GAPDH band intensity, (**b**) LTF band intensity relative to GAPDH band intensity, and (**c**) PIGR band intensity relative to GAPDH band intensity on the western blots from Figure 1g and 1h (n=5). Data represent the mean ± SD. Statistical significance was determined using an unpaired Student's *t* test and is indicated as *, $p \leq 0.05$; **, $p \leq 0.01$. **d-h,** Transcript levels of (**d**) *S100a8*, (**e**) *S100a13*, (**f**) *Reg3d*, (**g**) *C3*, and (**h**) *Defb1* from 7-day old infant mice and adult mice (n=8-11). Data represent $\log_2$-transformed values and the mean ± SD. Statistical significance was determined using an unpaired Student's *t* test and is indicated as *, $p \leq 0.05$; ****, $p \leq 0.0001$. Data collected from two independent experiments.

S Figure 2



a, Individual colonization levels of *S. pneumoniae* wild-type and *pgdA* mutant strains from competitive infections in wild-type infant and adult mice at 7 days post-infection from Figure 2b (n=4-7). **b**, Individual colonization levels of *S. pneumoniae* wild-type and *pgdA* mutant strains from competitive infections in wild-type and lysozyme 2-deficient infant mice at 3 and 14 days post-infection from Figure 2e (n=4-20). **c**, Transcript levels of *Lyz1/2* in nasal lavages collected from naïve adult wild type and lysozyme-2 deficient mice (n=4-6). Data represent log_2-transformed values and the mean ± SD. Statistical significance was determined using an unpaired Student's *t* test and is indicated as ****, $p \leq 0.0001$. Data collected from one experiment.

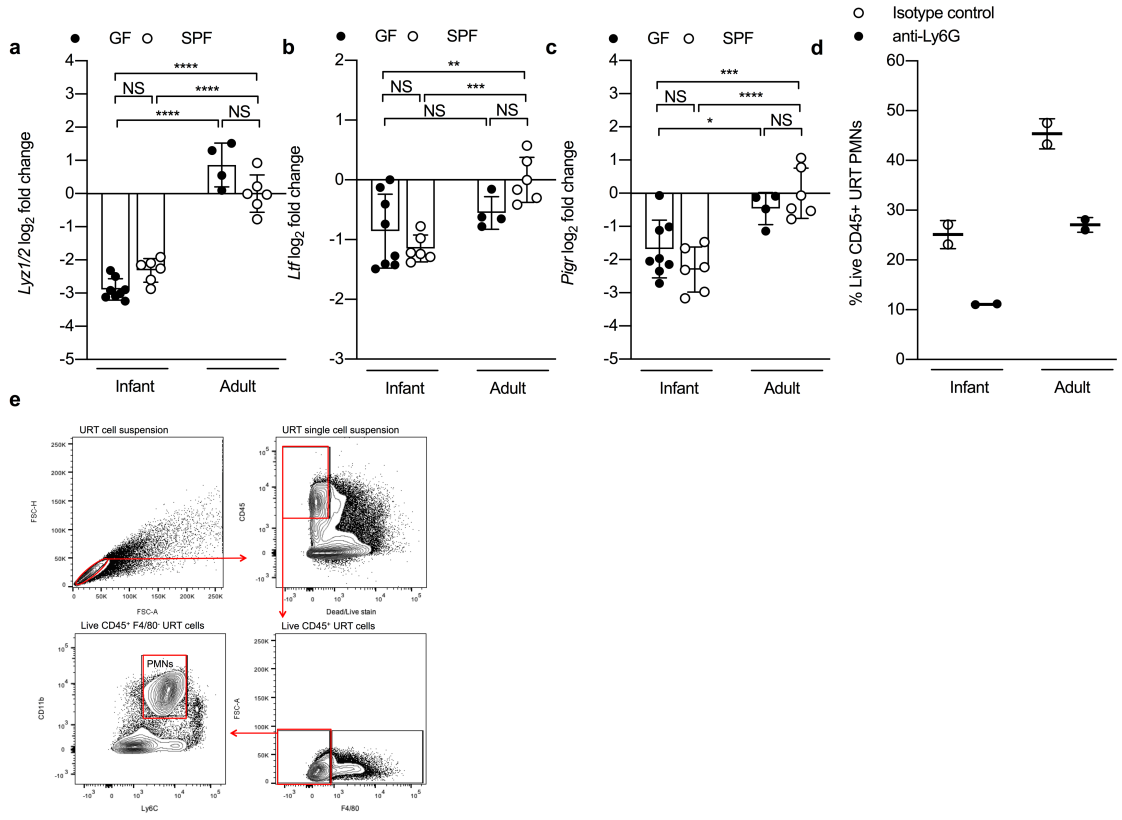
S Figure 3



a, Densitometric quantification of LYZ1/2 band intensity relative to GAPDH band intensity on western blot from Figure 3b (n=4). Data represent the mean ± SD. Statistical significance was determined using an unpaired Student's *t* test and is indicated as **, $p \leq 0.01$. **b,** Individual colonization levels of *S. aureus* wild-type and *oatA* mutant strains from competitive infections in wild-type infant mice at 7, 14 and 28 days post-infection from Figure 3c (n=6-8). **c**, Transcript levels of *Ltf* in ileum lavages collected from infant mice at 6, 15, 21 and 28 days of age compared to adult mice (n=5-8). Data represent $\log_2$-transformed values with the mean ± SD. **d**, Densitometric quantification of PIGR band intensity relative to GAPDH band intensity on western blot from Figure 3e (n=4). Data represent the mean ± SD. Statistical significance was determined using an unpaired Student's *t* test and is indicated as **, $p \leq 0.01$. **e**,

Transcript levels of *Reg3g* in ileum lavages collected from infant mice at 6, 15, 21 and 28 days of age compared to adult mice (n=5-8). Data represent log_2-transformed values with the mean ± SD. Statistical significance for *Ltf* and *Reg3g* transcripts was determined using an ordinary one-way ANOVA and Dunnett's multiple comparisons post-hoc test and is indicated as *, $p \leq 0.05$; *****, $p \leq 0.0001$. Data collected from two independent experiments, except D15 data collected from one experiment.

S Figure 4



a-c, Transcript levels of (**a**) *Lyz1/2* (**b**) *Ltf* and (**c**) *Pigr* in nasal lavages collected from naïve germ free (GF) and specific pathogen free (SPF) infant (6-9 day old) and adult mice. Data represent log_2-transformed values with the mean ± SD. Statistical significance was determined using an ordinary one-way ANOVA and Sidak's multiple comparisons post-hoc test and is indicated as **, $p \leq 0.01$; ***, $p \leq 0.001$; *****, $p \leq 0.0001$. NS, not significant. Data collected from two independent experiments, except GF adult samples collected from one experiment. **d**, Frequency of URT neutrophils (singlet live $CD45^+$ $F4/80^-$ $CD11b^+$ $Ly6C^+$) from infant and adult mice treated with 2 doses of either a neutrophil depleting antibody (αLy6G) or isotype control (n=2). Data represent mean ± SD. Data collected from one experiment. **e**, Gating strategy for defining the neutrophil population in the URT after treatment with either a neutrophil

depleting antibody (αLy6G) or isotype control. Neutrophils defined as singlet live $CD45^{+}$ $F4/80^{-}$ $CD11b^{+}$ $Ly6C^{+}$. PMNs, neutrophils.

Supplemental methods

Gnotobiotic mice

Germ-free C57BL/6J were purchased from Jackson Laboratory and were bred and maintained in flexible film isolators. The absence of fecal bacteria and fungi was confirmed by aerobic culture in brain heart infusion, sabaraud, and nutrient broth; and qPCR for bacterial 16S and eukaryotic 18S ribosomal RNA genes stool collected from individual cages in each isolator on a monthly basis. For experiments adult male and female (8-12 week-old) and infant (6-9 day old) mice were used.

Mouse infections

Infant mice (5-7 days-old) were infected with $1x10^3$ CFU of *S. pneumoniae* in 3ul of sterile phosphate-buffered saline (PBS) by intranasal instillation. Adult male and female mice (7-10 weeks-old) were infected with 10^5-10^6 CFU of *S. pneumoniae* in 10ul of sterile PBS by intranasal instillation. For competition experiments, infant and adult mice were infected with *S. pneumoniae* and *S. pneumoniae pgdA* mutant at a 1:1 ratio of either $1x10^3$ or $1x10^5$ CFU, respectively. All mice were euthanized by CO_2 asphyxiation followed by cardiac puncture. To assess URT bacterial load, the trachea was cannulated and lavaged with 200-400µl of sterile PBS. Samples were subsequently serially diluted 10-fold and 10µl, droplets were plated in triplicate on TS plates supplemented with appropriate antibiotics. For competition experiments, wild-type bacteria was calculated by subtracting the number of colonies that grew on the TS+kanamycin plates from the number of colonies on the TS+neomycin plates. The

competitive index was calculated by taking the ratio of both strains recovered from the sample and divided by the inoculum ratio.

For *S. aureus* experiments, infant mice (7-days-old) were infected with *S. aureus* and a *S. aureus oatA* mutant at a 1:1 ratio of $2x10^7$ CFU. Infant mice were mouth-fed with a total of $4x10^7$ CFU of *S. aureus* suspended in 10µl of filter sterilized PBS with 20% sucrose (FischerScientific). All mice were euthanized by CO_2 asphyxiation followed by cardiac puncture. To assess cecal *S. aureus* levels, the cecum was collected and suspended in 1ml of sterile PBS followed by homogenization using a Tissue Tearor Model 398 homogenizer (Biospec Products). Samples were serially diluted 10-fold and 100µl was plated on CHROMID MRSA SMART II agar plates. A minimum of 100 colonies recovered from CHROMID MRSA SMART II agar plates were replica-plated to TS and TS+5µg/ml erythromycin plates to distinguish between the wild-type and *oatA* mutant strains. Amount of wild-type bacteria was calculated by subtracting the number of colonies that grew on the TS+erythromycin plates from the number of colonies on the CHROMID MRSA SMART II plates. The competitive index was calculated by taking the ratio of both strains recovered from the sample and divided by the inoculum ratio.

RNA isolation and Real-Time PCR

At the indicated time-points, naïve infant and adult mice were euthanized by CO_2 asphyxiation followed by cardiac puncture. To obtain RNA from the URT, the trachea was cannulated and lavaged with 200 µl of PBS and then with 600µl of RLT lysis buffer (QIAGEN;) containing 1% 2-mercaptoethanol (Sigma). To obtain RNA from the ileum of

the small intestine, a cannulated needle was used to lavage 1 ml of PBS through the lumen to remove lumenal contents. A second lavage of 600µl of RLT lysis buffer (QIAGEN) containing 1% 2-mercaptoethanol (Sigma) was used for RNA collection. RNA was isolated using a QIAshredder kit (QIAGEN) followed by an RNeasy minikit (QIAGEN) per the manufacturer's protocol. Total RNA was reverse transcribed using a High-Capacity cDNA Reverse Transcription Kit (ThermoFisher). cDNA was purified using a MiniElute PCR Purification Kit (QIAGEN) according to manufacturer's protocol, except cDNA was eluted in 35µl of UltraPure™ DNase/RNase-free water (Invitrogen). After elution cDNA was diluted with 80µl of DNase/RNase-free water. Real-time PCR was performed using 4µl of cDNA, 0.625µl of each gene specific primer (10µM), 12.5µl of SYBR green (Applied Biosystems) and 7.25µl of DNase/RNase-free water per sample using a CFX384 Touch™ Real-Time PCR Detection System (BioRad). Target genes were normalized to respective *Gapdh* levels and data represent fold change according to the ΔΔCt method. Primer sequences for genes can be found in Supplementary table 6.

RNA-Sequencing

Infant (4-day-old) and adult (8-week-old) mice were mock-treated with 3µl or 10µl, respectively, of PBS and were euthanized by CO_2 asphyxiation followed by cardiac puncture 3-days post-treatment. Mouse tracheas were lavaged with 200ul of PBS followed by a second lavage with 600ul of RLT lysis buffer to collect RNA. RNA was extracted using the QIAshredder kit followed by an RNeasy minikit, as described above. RNA quality was checked using a bioanalyzer, and five samples/group were used for

RNA-sequencing. For library preparation, the Illumina TruSeq Stranded mRNA Library Prep Kit (Illumina) was used according to the manufacturer's protocol. A total of 350ng RNA/sample with 11 cycles for final amplification was performed. Sequencing was performed using Illumina Hi-seq and the raw fastq reads were aligned to mm10 mouse references genome using STAR aligner[53]. Fastq Screen was used to check for any contamination in the samples and Picard RNA-seq Metrics was used to obtain the metrics of all aligned RNA-seq reads. *featureCounts*[54] was used to quantify the gene expression levels. Raw gene counts were used for differential expression analysis. To identify DEGs, *DESeq2* R package[55] was used and DEGs were analyzed using online annotation tool PANTHER Bioinformatics Resources[56]. Heatmaps were generated using *pheatmap* R package. RNA-seq data are made available in the GEO repository under project accession number GSE116604.

URT and small intestine protein isolation and western blots

Protein from the upper respiratory tract was collected from naïve infant and adult mice. Mice were euthanized by CO_2 asphyxiation followed by cardiac puncture. To collect protein, the trachea was cannulated and lavaged with 600 µl of Pierce™ RIPA lysis buffer (ThermoFisher; cat. 89901) containing cOmplete™ Mini EDTA-free Protease Inhibitor Cocktail (Roche; cat. 1183670001). Protein was quantified using the Pierce BCA Protein Assay Kit (ThermoFisher; cat. 23227) according to the manufacturer's protocol and was normalized to 65-100 ug/ml.

Protein from the small intestine, which included the duodenum, jejunum and ileum, was collected from naïve infant and adult mice. Mice were euthanized by CO_2 asphyxiation followed by cardiac puncture. To collect protein, the entire small intestine was removed and lavaged with 5 ml ice-cold Dulbecco's phosphate-buffered saline (dPBS) (Gibco; cat. 14190-144). The small intestine was then cut open longitudinally, placed in a petri dish containing ice-cold dPBS to further remove fecal matter, cut into multiple pieces and placed in 10 ml of dissociation reagent #1, [47 ml dPBS, 3 ml 0.5 M ethylenediaminetetraacetic acid (EDTA; Invitrogen; cat. 15575-038) and 75 µl of 1 M dithiothreitol, (DTT; Sigma-Aldrich; cat. D0632),] and incubated on ice for 20 minutes. Tissue was removed with forceps and placed in 6 ml of dissociation buffer #2, [47 ml dPBS and 3 ml of 0.5 M EDTA warmed to 37°C] and incubated at 37°C for 10 minutes. The tissue was vigorously shaken for 30 seconds, at 2.5-3 shake cycles per second. Remnant small intestinal tissue was removed with forceps and cell solution was centrifuged at 800 x g for 5 minutes at 4°C. Dissociated cells were washed by removing supernatant and re-suspending cells in 10 ml of dPBS. The cell solution was centrifuged at 800 x g for 5 minutes at 4°C, supernatant was removed, and the cells were re-suspended in 1 ml of ice-cold RIPA buffer with protease inhibitor cocktail.

For western blots, approximately 26 µl of each sample was loaded and separated under denaturing conditions using Bolt™ 4%-12% Bis-Tris Plus gels (Invitrogen; cat. NW04122BOX) and transferred onto iBlot™ 2 Transfer Stacks nitrocellulose membranes (Invitrogen; cat. IB23002) using a dry blotting system (iBlot 2, ThermoFisher Scientific). The blot was incubated overnight in a blocking solution of

2.5% bovine serum albumin (BSA; Sigma; cat. A9430) and 0.1% Tween-20 (Sigma; cat. P9416) in PBS. After washing 3 times with 0.1% Tween-20/PBS, proteins were detected with either a polyclonal rabbit anti-human lactoferrin antibody (1:5,000; Sigma; cat. L-3262), polyclonal rabbit anti-mouse lysozyme [1:500; provided by Henry T. Akinbi, University of Cincinnati College of Medicine], goat anti-mouse pIgR polyclonal antibody (1:5,000; R&D; cat. AF2800), or a rabbit anti-GAPDH polyclonal antibody (1:10,000; ThermoFisher Scientific; cat. PA5-85074). Primary antibodies were diluted in 0.1% Tween-20/PBS and incubated with membranes at room temperature for 2 hours. For detection of lysozyme, lactoferrin, or GAPDH, a goat anti-rabbit horseradish peroxidase (HRP)-conjugated secondary antibody (ThermoFisher; cat. G-21234) was diluted 1:20,000 in 0.1% Tween-20/PBS and applied to the membrane for 45 minutes at room temperature. For detection of the pIgR, a rabbit anti-goat HRP-conjugated secondary antibody (R&D; cat. HAF017) was diluted either 1:10,000 for URT samples or 1:20,000 for small intestine samples in 0.1% Tween-20/PBS and applied to the membrane for 45 minutes at room temperature. Protein bands were detected by chemiluminescence using SuperSignal™ West Pico PLUS Chemiluminescent Substrate (ThermoFisher; cat. 34577) and visualized using an iBright CL1000 Imaging System (ThermoFisher). Densitometry was calculated using the local corrected density value from each band determined by the iBright CL1000 Imaging System. Values for target proteins were normalized to GAPDH values.

Flow cytometry

198 Flow cytometry analysis was performed to characterize the cellular composition of
199 mouse nasal tissue. Infant and adult mice were euthanized by CO_2 asphyxiation
200 followed by cardiac puncture. To isolate nasal tissue cells, heads were detached and
201 the skin, lower jaw, tongue, eyes, and incisors were removed. The skull was cut
202 laterally, starting behind the ears and the posterior skull, and brain tissues were
203 removed. The remaining tissue was cut into small pieces and digested with collagenase
204 type II (200 U/ml; ThermoFisher; cat. 17101015) and DNase (15 U/ml; Sigma-Aldrich;
205 cat. 4716728001) in DMEM media (Gibco; cat. 11995-065) for 1 hour at 37°C on a
206 rocker. Tubes were shaken vigorously for 30 seconds every half hour during incubation.
207 Cells were passed through a 70 µm filter and centrifuged for 10 min at 500 x g at 4°C.
208 Excess debris was removed using a Debris Removal Solution (Miltenyi Biotec; cat. 130-
209 109-398) according to the manufacturer's protocol. Once free from debris, cells were
210 treated with ACK lysing buffer (Lonza; cat. 10-548E) for 5 min to lyse red blood cells,
211 then washed with dPBS and centrifuged for 10 min at 500 x g at 4°C. The pellet was re-
212 suspended in dPBS and cells were passed through a 40 µm filter; the suspension was
213 centrifuged for 10 min at 500 x g at 4°C. Single nasal cell suspensions were re-
214 suspended in dPBS and stained with a Live/Dead™ Fixable Green Dead Cell Stain Kit
215 (ThermoFisher; cat. L34969) according to the manufacturer's protocol. After Live/Dead
216 staining, cells were washed with dPBS and re-suspended in 50 ul of fluorescence-
217 activated cell sorter (FACS) buffer (dPBS containing 1% BSA and 2 mM EDTA). Cells
218 were stained with a FC receptor-blocking antibody, anti-CD16/32 (BioLegend; cat.
219 101301; clone 93) for 5 minutes at 4°C and then stained with a cocktail of anti-CD45
220 APC-Cy7 (BD; cat. 557659; clone 30-F11), anti-F4/80 PE (BioLegend; cat. 123110;

clone BM8), anti-CD11b V450 (BD; cat. 560455; clone M1/70) and anti-Ly6G PerCp/Cy5.5 (BD; cat. 560602; clone 1A8) for 30 minutes at 4°C. Cells were washed in FACS buffer, fixed with 4% paraformaldehyde (Affymetrix; cat. 19943) for 30 minutes at 4°C and then re-suspended in 50 ul of FACS buffer. Flow cytometry analysis was performed using a LSRII apparatus (Becton Dickinson) and approximately 1.5-3 x 10^5 total events were acquired for each sample. Resulting data was analyzed using FlowJo software (Treestar inc., Ashland, OR). Gates were based on Fluorescence-Minus-One (FMO) controls.

URT and small intestine CD45+/- cell isolation

Single cell suspensions from nasal tissue were obtained using the method described above. Cell suspensions from the small intestine were obtained using the method described above and the following steps were used to obtain a single cell suspension. Once dissociated cells were washed with dPBS and pelleted at 800 x g for 5 minutes at 4°C; the supernatant was removed and cells were re-suspended in 5 ml TryPLE express (Gibco, cat. 12604-013). The cell suspension was incubated for 20 minutes at 37°C on a rocker and were shaken vigorously every 5-10 minutes. 5 ml of 10% fetal bovine serum (FBS; Gibco, cat. 10082147) in dPBS was added and the solution was mixed by gently pipetting up and down. Cells were pelleted at 800 x g for 5 minutes at 4°C and debris was removed using Debris Removal Solution. Cells were centrifuged at 800 x g for 5 minutes at 4°C, the supernatant was removed and the pellet was re-suspended in 1 ml of ACK lysis buffer. Cells were incubated for 5 minutes at room temperature and then washed with 10 ml of 10% FBS in dPBS. Cells were centrifuged

at 800 x g for 5 minutes at 4°C to produce a single cell suspension. Once single cell suspensions were obtained, dead cells were excluded using a Dead Cell Removal Kit (Miltenyi Biotech; cat. 130-090-101). Cells were isolated using CD45 mouse MicroBeads (Miltenyi Biotec; cat. 120-052-301) and MS columns (Miltenyi Biotech; cat. 130-042-201) according to the manufacturer's protocol. Once collected $CD45^+$ and $CD45^-$ cells were suspended in RLT lysis buffer and RNA was isolated as described above.

***In vivo* neutrophil depletion**

For neutrophil depletion experiments, infant or adult mice were injected intraperitoneally with either 150 ug or 500 ug, respectively, of either rat anti-mouse Ly6G monoclonal antibody (BioXcell; cat. BE0075-1; clone 1A8) or rat IgG2a isotype control (BioXcell; cat. BE0089; clone 2A3;) diluted in 30 ul of dPBS for infant mice or 100 ul of dPBS for adult mice. The antibodies were administered twice at 48 hours apart and mice were euthanized 24 hours after the second dose. Neutrophil depletion in the upper respiratory tract was confirmed by flow cytometry as described above.

Immunohistochemistry,

Naïve infant (6 day old) and adult (8 week old) mice were euthanized by CO_2 asphyxiation followed by cardiac puncture. The animals were decapitated and after removal of the skin, the head was fixed for 48 hours in 4% paraformaldehyde. The head was then decalcified by incubation in either 0.12 M EDTA (pH 8.0) for 1 week for infant heads or 0.25 M EDTA (pH 8.0) for 3 weeks for adult heads shaking at 4°C with daily

changes of the EDTA solution. Heads were then processed through graded ethanols to xylene and infiltrated with paraffin in a Leica Peloris automated tissue processor. Five µm paraffin sections were stained with hematoxylin and eosin or immunostained with an antibody against mouse pIgR (R&D, Cat #AF2800) or mouse lactoferrin (Sigma, cat # L3262) with the Leica DAB KIT (Cat #DS9800) on a Leica BondRX autostainer, according to the manufacturer's instructions. In brief, sections underwent 20-minute heat-induced epitope retrieval with Leica ER2 buffer (pH 9, Cat. AR9640) prior to a 30-minute incubation with either rabbit anti-lactoferrin (1:100 dilution) or goat anti-pIgR (1:100 dilution). Primary antibody incubations were followed with Leica anti-rabbit (Leica DAB kit, undiluted, 8 minutes) or Goat Probe and Goat-on-Rodent horseradish peroxidase-coupled polymers (Biocare, Cat #GHP516 H, undiluted, 10 minutes each) and the substrate diaminobenzidine (Leica DAB kit, Cat DS9800, 10 minutes). Slides were counterstained with hematoxylin and imaged on a Hamamatsu Nanozoomer whole slide scanner.

Human study

Details on study design and inclusion criteria were published previously[57,58]. Briefly, non-respiratory tract infection (RTI) nasopharyngeal samples were collected from 112 healthy infants at up to 11 time-points (2 hours after birth, at 24 hours, at 7 and 14 days, and at 1, 2, 3, 4, 6, 9, and 12 months of age). For the study, we selected the final 43 inclusions (*n*=286 samples), which were stored in RNA protect Cell Reagent (Qiagen) at -80 °C. RNA extraction was performed by the TRIzol/chloroform method, subsequently

290 cleaning samples with the RNAeasy Micro Kit (Qiagen)(OpenWetWare contributors,
291 2015). Quality and quantity of RNA was assessed using 2100 Bioanalyzer (Agilent).
292 Further processing of samples was done by Hologic Ltd. (Manchester UK); cRNA was
293 prepared and hybridized onto Affymetrix Clariom S Human Pico arrays.

294 Bio-informatic quality control was performed as previously described[59]. Following,
295 robust multichip averaging (RMA) background correction and quantile normalization
296 was performed. Log_2-transformed gene expression values were used for downstream
297 analyses. Statistical analyses were performed in R version 4.0.2 within R studio version
298 1.4.623 (Boston, MA). Gene expression data are available upon request.

S Table 1: Normalized and non-normalized densitometry values for upper respiratory tract (URT) lysozyme western blot (Figure 1g and Supplementary Figure 1a).

Mouse ID and tissue	Local corrected density		GAPDH normalization factor	Lysozyme over normalized GAPDH factor
	Lysozyme	GAPDH		
Infant 1 URT	156	5,447	0.458	340.448
Infant 2 URT	632	8,363	0.703	898.925
Infant 3 URT	568	6,620	0.556	1020.930
Infant 4 URT	679	5,549	0.466	1456.263
Infant 5 URT	74	6,174	0.519	143.077
Adult 6 URT	5,745	4,272	0.359	15999.754
Adult 7 URT	2,053	4,303	0.362	5675.337
Adult 8 URT	7,309	11,898	1.000	7308.790
Adult 9 URT	6,718	8,583	0.721	9312.962
Adult 10 URT	1,005	4,411	0.371	2711.239

S Table 2: Normalized and non-normalized densitometry values for upper respiratory tract (URT) lactoferrin western blot (Figure 1g and Supplementary Figure 1b).

Mouse ID and tissue	Local corrected density		GAPDH normalization factor	Lactoferrin over normalized GAPDH factor
	Lactoferrin	GAPDH		
Infant 1 URT	1,184	5,447	0.458	2586.135
Infant 2 URT	539	8,363	0.703	766.886
Infant 3 URT	933	6,620	0.556	1676.158
Infant 4 URT	1,018	5,549	0.466	2183.581
Infant 5 URT	67	6,174	0.519	129.674
Adult 6 URT	16,444	4,272	0.359	45793.868
Adult 7 URT	4,891	4,303	0.362	13524.154
Adult 8 URT	15,457	11,898	1.000	15456.700
Adult 9 URT	12,015	8,583	0.721	16655.117
Adult 10 URT	9,621	4,411	0.371	25951.218

S Table 3: Normalized and non-normalized densitometry values for upper respiratory tract (URT) PIGR western blot (Figure 1h and Supplementary Figure 1c).

Mouse ID and tissue	Local corrected density		GAPDH normalization factor	PIGR over normalized GAPDH factor
	PIGR	GAPDH		
Infant 1 URT	199	11,054	1.000	199.368
Infant 2 URT	667	8,129	0.735	907.033
Infant 3 URT	40	5,682	0.514	78.432
Infant 4 URT	1,296	6,428	0.582	2229.083
Infant 5 URT	318	4,541	0.411	774.662
Adult 6 URT	3,078	3,328	0.301	10225.124
Adult 7 URT	6,011	3,386	0.306	19623.945
Adult 8 URT	1,818	10,220	0.925	1966.203
Adult 9 URT	18,077	5,997	0.542	33322.239
Adult 10 URT	14,618	6,119	0.554	26406.386

S Table 4: Normalized and non-normalized densitometry values for small intestine (SI) lysozyme western blot (Figure 3b and Supplementary Figure 3c).

Mouse ID and tissue	Local corrected density		GAPDH normalization factor	Lysozyme over normalized GAPDH factor
	Lysozyme	GAPDH		
Infant 1 SI	83	11,142	0.599	138.367
Infant 2 SI	430	7,984	0.430	1001.319
Infant 3 SI	145	7,729	0.416	348.622
Infant 4 SI	110	8,130	0.437	251.632
Adult 1 SI	7,915	8,714	0.469	16882.289
Adult 2 SI	7,780	11,019	0.593	13124.925
Adult 3 SI	6,615	15,456	0.832	7954.904
Adult 4 SI	7,852	18,588	1.000	7851.690

315 **S Table 5:** Normalized and non-normalized densitometry values for small intestine
316 PIGR western blot (Figure 3e and Supplementary Figure 3e).
317

Mouse ID	Local corrected density		GAPDH normalization factor	PIGR over normalized GAPDH factor
	PIGR	GAPDH		
Infant 1 SI	1,840	6,497	0.298	6176.536
Infant 2 SI	2,986	4,901	0.225	13286.501
Infant 3 SI	1,273	7,154	0.328	3881.251
Infant 4 SI	86	4,586	0.210	411.168
Adult 1 SI	11,806	9,395	0.431	27407.622
Adult 2 SI	13,388	10,561	0.484	27649.649
Adult 3 SI	10,577	14,102	0.647	16359.001
Adult 4 SI	22,798	21,810	1.000	22798.000

318

S Table 6: Quantitative real-time PCR (qRT-PCR) primers used in this study.

Target	Species	Primer sequences (5'-3')	
		Forward	Reverse
Gapdh	*Mus musculus*	TCATCAACGGGAAGCCCA	AGACTCCACGACATACTCA
Lyz1/2	*Mus musculus*	TGAAGACTCTCCTGACTC	ACGGTTGTAGTTTGTAGC
Ltf	*Mus musculus*	TGCTTGCTAACCAGACCAGA	ACCAATACACAGGGCACAGA
Pigr	*Mus musculus*	ATGAGGCTCTACTTGTTCACGC	CGCCTTCTATACTACTCACCTCC
S100a8	*Mus musculus*	TGTCCTCAGTTTGTGCAGAATATAAA	TCACCATCGCAAGGAACTCC
S100a13	*Mus musculus*	AACTGCCTCATTTGCTCAAGG	AGTCTCCAGTATTCACTGAACCT
Reg3d	*Mus musculus*	ACCACAGACCTGGGCTAATG	AGTCCAATCCAGATGTATGGGAA
C3	*Mus musculus*	CCAGCTCCCCATTAGCTCTG	GCACTTGCCTCTTTAGGAAGTC
Sftpd	*Mus musculus*	CAAAAGGCTCCACAGGCCCCA	CAGCACTGTCTGGAAGCCCGC
Defb1	*Mus musculus*	AGGTGTTGGCATTCTCACAAG	GCTTATCTGGTTTACAGGTTCCC
Reg3g	*Mus musculus*	ATGGCTCCTATTGCTATGCC	GATGTCCTGAGGGCCTCTT