Commensal Bacteroidetes protect against *Klebsiella pneumoniae* colonization and transmission through IL-36 signalling

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1   **The microbiota primes immune defences but the identity of specific commensal microbes which**

2   **protect against infection is unclear. Conversely, how pathogens compete with the microbiota to**

3   **establish their host niche is also poorly understood. Here, we investigate the antagonism**

4   **between the microbiota and *Klebsiella pneumoniae* during colonization and transmission. We**

5   **discover that maturation of the microbiota drives the development of distinct immune defence**

6   **programs in the upper airway and intestine to limit *K. pneumoniae* colonization within these**

7   **niches. Immune protection in the intestine depends on the development of Bacteroidetes, IL-36**

8   **signalling and macrophages. This effect of Bacteroidetes requires their conserved commensal**

9   **colonization factor (CCF) polysaccharide utilization locus. Conversely, in the upper airway,**

10   **Proteobacteria prime immunity through IL-17A, but *K. pneumoniae* overcomes these defences**

11   **through encapsulation to effectively colonize this site. Ultimately, we find that host-to-host**

12   **spread of *K. pneumoniae* occurs principally from its intestinal reservoir, and that CCF-producing**

13   **Bacteroidetes are sufficient to prevent transmission between hosts through IL-36. Thus, our**

14   **study provides mechanistic insight into when, where and how commensal Bacteroidetes protect**

15   **against *K. pneumoniae* colonization and contagion, providing insight into how these protective**

16   **microbes could be harnessed to confer population-level protection against *K. pneumoniae***

17   **infection.**

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INTRODUCTION

The microbiota enhances immune defences to protect against pathogenic microbes[1-4]. Identifying members of the microbiota that protect against pathogens could therefore provide an alternative means to treat infections resistant to current antimicrobial therapies[5]. Of these antimicrobial resistant organisms, it is bacteria from the *Enterobacteriaceae* family which are the greatest clinical problem[6-9]. Within this family, *K. pneumoniae* are the most urgent threat to human health because many strains are resistant to multiple antibiotics, are highly virulent, cause disease in both adults and infants, and readily spreading between hosts[8,10-14].

Asymptomatic colonization is the pivotal step in *K. pneumoniae* pathogenesis as this serves as the reservoir of organisms which initiate acute infection and is also the source of organisms which transmit to other hosts[10-13,15,16]. *K. pneumoniae* is a versatile colonizer with two principal niches within the human host: the upper airway and intestine[11,13,15,17,18]. To establish colonization *K. pneumoniae* must therefore contend with defences established by the microbiota and immune system at both of these sites. Clinical data confirm the importance of the microbiota and immune system in resisting the establishment of *K. pneumoniae* colonization. Specifically, the association between patients receiving antibiotics and increased incidence of *K. pneumoniae* colonization[10,19]; the demonstration that microbiota transplantation, in humans and mice, clears *K. pneumoniae* from the intestine[20-24]; and the association between immunosuppression and increased risk of *K. pneumoniae* colonization[25-27] highlight the critical role of the microbiota and immune system in protecting against *K. pneumoniae* colonization. The mechanistic basis for this protection, and whether there is collaboration between these microbial and immune defences system, is poorly understood hampering the use of microbiota-based approaches to protect against *K. pneumoniae.*

Once colonization has been established, these hosts serve as reservoirs for the transmission of *K. pneumoniae* within a population. The process of transmission involves exiting the colonized host,

surviving in the environment, acquisition by a new host, and then successful reestablishment of colonization at a new mucosal site. Although the importance of *K. pneumoniae* transmission is undoubted, and has underpinned its success as a pathogen, the host and bacterial factors dictating the outcome of transmission are poorly defined. Here, by analysing the development of microbiota-mediated defences in the upper airway and intestine, we reveal the commensal taxa that inhibit *K. pneumoniae* colonization and transmission, the immunological basis for their protection, and the countervailing tactics used by *K. pneumoniae* to establish colonization in the face of these defences. Through this fundamental analysis, we define a simple consortium of commensal microbes which prevent the host-to-host spread of this contagious pathogen.

1 **RESULTS**

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3 **The development of the adult microbiota protects against colonization by antibiotic-resistant**

4 *Klebsiella pneumoniae* **in the intestine but not upper airway**

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6 As *K. pneumoniae* causes disease in infants and adults, we wanted to investigate the development of

7 microbiota-mediated defences against *K. pneumoniae* colonization across lifecourse. To do this, we

8 depleted the microbiota in neonatal and adult mice (**Supplementary Fig. 1a-e**). To study upper airway

9 colonization by *K. pneumoniae*, we used a small inoculation volume and did not anaesthetize mice for

10 intranasal inoculation. This approach means bacteria remain in the nasal cavities and nasopharynx and

11 do not reach the lung[28,29] (**Supplementary Fig. 2**). In neonates, *K. pneumoniae* was able to colonize

12 both the upper airway and intestine and this unaffected by microbiota-depletion (**Fig. 1a-c**). Likewise, in

13 the adult upper airway, *K. pneumoniae* colonization levels were similar between control and microbiota-

14 depleted animals (**Fig. 1d-f**). In the adult intestine, by contrast, *K. pneumoniae* could only establish

15 detectable colonization after microbiota-depletion (**Fig. 1g-j**). Sustained broad spectrum antibiotic

16 treatment (**Fig. 1g-j**), or a short, clinically-relevant antibiotic regimen (**Supplementary Fig. 3a,b**)

17 eliminated microbiota-mediated defences. Transferring the adult, but not the neonatal microbiota to

18 neonatal mice protected against intestinal colonization by *K. pneumoniae* (**Fig. 1k-m**). The adult

19 intestinal microbiota was broadly protective, inhibiting colonization by clinical isolates of *K. pneumoniae*

20 producing the OXA-48 carbapenenemase (**Fig. 1g**), the pandemic *K. pneumoniae* ST258 (**Fig. 1l**), and

21 closely related *K. quasipneumoniae subsp. similipneumoniae* (**Fig. 1j**). No weight loss was observed

22 during colonization of either niche (**Supplementary Fig. 4a,b**) supporting the notion that we were

23 modelling asymptomatic colonization. These data show that the development of the adult microbiota

24 creates a barrier sufficient to block *K. pneumoniae* colonization in the intestine but not the upper airway.

25 This raised three questions: (i) which members of the adult intestinal microbiota prevent *K. pneumoniae*

26 colonization, and by what mechanism? (ii) What factors allow *K. pneumoniae* to successfully compete

27 with the upper airway microbiota to colonize this niche? And, most critically, (iii) how does this

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competition between the microbiota and *K. pneumoniae* at different mucosal reservoirs impact the host-to-host spread of this contagious pathogen?

The development of Bacteroidetes prevents *Klebsiella pneumoniae* intestinal colonization

To answer our first question, we sequenced the neonatal and adult mouse intestinal microbiota to determine the respective composition of these permissive and inhibitory microbial communities. The neonatal intestinal microbiota was dominated by bacteria from the Firmicutes phylum, and specifically members of the Lactobacillales order (**Fig. 2a,b**). The adult intestinal microbiota, by contrast, was composed predominately of commensals from the Bacteroidetes (Bacteroidales) and Firmicutes (Clostridia and Lactobacillales) phyla, with Actinobacteria and Proteobacteria comprising a minor fraction of the commensal taxa present (**Fig. 2a,b**). We therefore used defined consortia of commensals to determine whether the development of Bacteroidetes, non-Lactobacillales Firmicutes, Actinobacteria, or Proteobacteria drove resistance to intestinal colonization by *K. pneumoniae*. We found that only Bacteroidetes promoted clearance of *K. pneumoniae* from the intestine, whether administered before or after the establishment of *K. pneumoniae* colonization (**Fig. 2c-k**). Bacteroidetes were protective in both antibiotic treated (**Fig. 2d-f,i,j**) and germ-free mice (**Fig. 2g**) and this was consistent using commensal consortia of different compositions (**Extended Data Fig. 1**). We verified the intestinal microbiota of mice administered our representative commensal consortia was dominated by that consortium and that colonization levels of each consortia were similar (**Extended Data Fig. 2**). Additionally, we found that Bacteroidetes specifically protected against intestinal colonization by *K. pneumoniae* in neonates (**Fig. 2k**), supporting the idea that it is the development of this commensal phylum that protects against *K. pneumoniae* in adults.

CCF-producing Bacteroidetes fortifies the intestinal immune barrier via IL-36 and macrophages to prevent *Klebsiella pneumoniae* intestinal colonization

Next, we wanted to determine whether the protective effect of Bacteroidetes required host immunity. We therefore transferred the microbiota to microbiota-depleted mice administered the immunosuppressive dexamethasone, or vehicle control, prior to *K. pneumoniae* colonization. Dexamethasone eliminated the inhibitory effect of the microbiota on *K. pneumoniae* colonization (**Extended Data Fig. 3a,b**), indicating that protection required immune signalling. Furthermore, we found that dexamethasone treatment of non-antibiotic treated mice made these normally resistant mice susceptible to colonization by *K. pneumoniae* (**Extended Data Fig. 3c-e**). Similarly, we found that protection conferred by Bacteroidetes required immune signalling (**Extended Data Fig. 3f,g**). To ensure that dexamethasone was acting on the immune system rather than disrupting the microbiota, we transferred the microbiota from dexamethasone or vehicle control treated mice, to microbiota-depleted mice and then examined the inhibitory effect on *K. pneumoniae* colonization. Both of these microbiota provided equivalent protection against *K. pneumoniae* colonization (**Supplementary Fig. 5**), demonstrating that dexamethasone does not eliminate protective commensals from the microbiota but instead inhibits the stimulatory effect of the microbiota on the immune system. Next, we sought to determine the immune factors which translate the effect of Bacteroidetes into resistance against *K. pneumoniae* colonization. In the intestine, the transition from neonate to adult was marked by the increased homeostatic expression of a number of cytokines important in maintaining host-microbial homeostasis (**Supplementary Fig. 6**). To determine whether the protective effect of Bacteroidetes was driven by these cytokine, we abrogated the signalling of cytokines whose homeostatic expression increased from neonates to adults in a microbiota-dependent manner. Protection against intestinal colonization by *K. pneumoniae* by Bacteroidetes was abrogated after treatment with an antibody targeting the IL-36 receptor signalling, but not IL-17A or IFNγ, (**Fig. 3a-e**). Supporting the role of IL-36, we found that treatment with recombinant IL-36γ promoted clearance of intestinal *K. pneumoniae* (**Fig. 3f-h**) and that intestinal IL-36γ production in microbiota-depleted animals was specifically restored by Bacteroidetes (**Fig. 3i**). Within the intestine, macrophages act as a critical innate interface with the microbiota, so we therefore investigated their role in protection against *K. pneumoniae* using liposome clodronate treatment to deplete these cells (**Supplementary Fig. 7**). After macrophage depletion,

Bacteroidetes could not protect against *K. pneumoniae*, or regulate IL-36γ (**Fig. 3j-m**). By contrast Bacteroidetes were still protective after neutrophil depletion (**Supplementary Fig. 8**). Furthermore, in clodronate treated mice, disruption of IL-36R signalling did not increase *K. pneumoniae* colonization, suggesting that IL-36 and macrophages work along a common pathway (**Fig. 3n**). To understand how IL-36 controls *K. pneumoniae* levels in the intestine via macrophages, we investigated if IL-36 promotes the bactericidal activity of these cells. We found that IL-36γ stimulation promoted the killing of multiple strains of *K. pneumoniae* by macrophages (**Extended Data Fig. 4a-d**). Next, we wanted to understand the factors in Bacteroidetes required for protection against *K. pneumoniae* colonization. Common amongst commensals that regulate intestinal immunity is a requirement for intimate contact with the host mucosa to exert their effects[30]. Highly conserved within Bacteroidetes is a capsular polysaccharide production locus, the commensal colonization factors (CCF), which promote the association of Bacteroidetes with the intestinal mucosa[31,32]. We tested the hypothesis that CCFs are required for Bacteroidetes to inhibit *K. pneumoniae* colonization. In agreement with this, a consortium of Bacteroidetes, or a single Bacteroidetes species, was able to protect against *K. pneumoniae* colonization and prime intestinal IL-36γ production with an intact CCF system, but this was lost in the absence of CCFs, and could be reestablished by complementation of the CCFs (**Fig. 4a-g**). In line with their proposed role in mucosal colonization, we found that the CCFs promoted the association of Bacteroidetes with the mucosa (**Fig. 4h**), but fecal levels of Bacteroidetes were equivalent in the presence or absence of CCFs (**Fig. 4i**). Together, our data support a model whereby CCF-producing Bacteroidetes associate with the mucosal barrier to create an immune barrier within the intestine preventing colonization by *K. pneumoniae* through IL-36 and macrophages.

The adult microbiota primes upper airway defences through IL-17A, but encapsulation allows *Klebsiella pneumoniae* to overcome this barrier to establish colonization

We then wanted to answer our second question: what factors allow *K. pneumoniae* to successfully compete with the upper airway microbiota to colonize this niche? To do this, we examined the role of *K.*

pneumoniae encapsulation. Traditionally, encapsulation has been viewed as a virulence determinant, however, capsular polysaccharide production is common to both pathogens and non-pathogens, especially in the upper airway[33]. In adult mice with a microbiota, we found that encapsulated *K. pneumoniae* colonized the upper airway higher levels than an isogenic unencapsulated *K. pneumoniae* mutant[34] and the ability to withstand microbiota-mediated defences was restored by complementation of the unencapsulated mutant (**Fig. 5a,b**). This advantage of encapsulation was lost in microbiota-depleted animals, where encapsulated and unencapsulated *K. pneumoniae* colonized to similar levels (**Fig. 5a,b**). Next, therefore, we determined whether encapsulation allows *K. pneumoniae* to withstand direct microbial competition within the upper airway, or indirect effects of the microbiota via the immune system. To address this, we transferred the microbiota back to microbiota-depleted animals treated with dexamethasone or vehicle control, prior to intranasal administration of *K. pneumoniae*. Microbiota transfer reduced upper airway colonization by unencapsulated *K. pneumoniae* in vehicle control (**Fig. 5a,b**), but not dexamethasone treated animals (**Fig. 5a,b**), supporting the notion that the microbiota enhances defences in the upper airway via immune signalling. In agreement with this, innate cytokine production (TNFα), which is induced by *K. pneumoniae* during upper airway colonization, was reduced in microbiota-depleted animals, and restored by microbiota transfer through immune signalling (**Extended Data Fig. 5a,b**). We confirmed that levels of upper airway commensal bacteria after microbiota transfer were equivalent between vehicle and dexamethasone treated mice (**Extended Data Fig. 5c**) and that microbiota transfer did not result in commensal inoculation into the lung (**Extended Data Fig. 6a**). Analogously to the intestine, microbiota priming of immune defences in the upper airway was specific to adult animals, as encapsulated and unencapsulated *K. pneumoniae* colonized the neonatal upper airway to similar levels and induced an equivalent innate cytokine response in microbiota-depleted and control animals (**Extended Data Fig. 7a,b**). The transition from neonate to adult was accompanied by the increased homeostatic expression of a number of cytokines in the upper airway which were dependent on the microbiota (**Supplementary Fig. 9**). We therefore abrogated signalling by these cytokines to understand if they were required for the adult microbiota to exert its immunomodulatory effects in the upper airway. Disruption of IL-17A, but not IFNγ or IL-36R signalling,

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1   inhibited microbiota-mediated clearance of unencapsulated, but not encapsulated, *K. pneumoniae* from

2   the upper airway (**Fig. 5c-f**)*. Furthermore, treatment with recombinant IL-17A was sufficient to promote

3   clearance of unencapsulated *K. pneumoniae* (**Fig. 5g,h**)*. The upper airway microbiota of mice

4   comprises bacteria from the Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria phyla

5   (**Extended Data Fig. 6b**)[35-41]. Of these commensals, we found that Proteobacteria specifically enhanced

6   clearance of unencapsulated *K. pneumoniae* from the upper airway in antibiotic treated and germ-free

7   mice by IL-17A signalling (**Fig. 5i-l**). Proteobacteria also restored homeostatic IL-17A production in the

8   upper airway and cytokine production induced by *K. pneumoniae* colonization (**Extended Data Fig. 8a-**

9   **c**). We verified the upper airway microbiota of mice administered our representative commensal

10 consortia was dominated by that consortium and colonization by each consortia were similar (**Extended**

11 **Data Fig. 6b**). These data support a model whereby Proteobacteria prime an IL-17A-dependent immune

12 defence program in the adult upper airway, however, encapsulation allows *K. pneumoniae* to withstand

13 these defences to successfully establish colonization.

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15 **Intestinal Bacteroidetes protect against *Klebsiella pneumoniae* transmission via IL-36 and this**

16 **requires the Bacteroidetes commensal colonization factors**

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18 Individuals colonized by *K. pneumoniae* act as reservoirs for its spread to other hosts within a

19 population[15,16,42,43] which led to our third question: how does the battle between *K. pneumoniae* and the

20 microbiota during colonization of different mucosal niches impact its host-to-host spread? To answer

21 this, first, we investigated the contribution of the upper airway and intestinal reservoirs to *K. pneumoniae*

22 transmission. To investigate the role of the upper airway reservoir, neonatal mice (7 – 11 days old) with

23 established upper airway *K. pneumoniae* colonization (referred to as "index" mice) were cohoused with

24 naïve (non-*K. pneumoniae* colonized) neonatal mice (referred to as "contact" mice) and then to

25 determine transmission, *K. pneumoniae* colonization levels in contact animals were quantified. After 4

26 days of cohousing there was no detectable *K. pneumoniae* in the upper airway of the contacts

27 (**Extended Data Figure 10, experiment 1**). No transmission was observed if the contact mice were

1   antibiotic treated or non-antibiotic treated before cohousing (**Extended Data Figure 10, experiment 2**).

2   There was, however, limited transmission from the upper airway of neonatal index mice to the intestine

3   of neonatal contacts (**Extended Data Figure 10, experiment 3**). This suggests that despite mice not

4   sneezing there is limited shedding of *K. pneumoniae* from the upper airway. Similarly to neonates, no

5   transmission was observed from the upper airway of adult index to the same niche in adult contact

6   animals (**Extended Data Figure 10, experiment 4**). We then assessed transmission between intestinal

7   reservoirs in neonates aged between 7 and 11 days as these animals are not coprophagic[44]. Cohousing

8   neonatal index mice with established intestinal colonization with naïve neonatal contacts resulted in

9   transmission of *K. pneumoniae* to the intestine of all contacts (**Extended Data Figure 10, experiment**

10   **5**), indicating that in the absence of microbiota-mediated defences, *K. pneumoniae* transmission occurs

11   primarily from the intestinal reservoir. We confirmed that the antibiotic resistance profiles of *K.*

12   *pneumoniae* isolated from neonatal contact mice matched the antibiotic resistance profiles of *K.*

13   *pneumoniae* inoculated into index mice (**Extended Data Fig. 9a**), confirming that the *K. pneumoniae*

14   isolated from contact mice originated from the index animals. To understand how the development of

15   the adult microbiota, which protects against *K. pneumoniae* colonization in the intestine, impacts

16   transmission, we used three approaches. Firstly, adult index mice with established intestinal *K.*

17   *pneumoniae* colonization were cohoused with either antibiotic treated, or non-antibiotic treated, contact

18   mice. Antibiotic treated adult contact animals all acquired *K. pneumoniae* (**Extended Data Figure 10,**

19   **experiment 6 and 7**), whereas there was limited transmission to non-antibiotic treated adult contact

20   animals (**Extended Data Figure 10, experiments 8 and 9**). Again, we confirmed that the antibiotic

21   resistance profiles of *K. pneumoniae* isolated from adult contact mice matched the antibiotic resistance

22   profiles of *K. pneumoniae* inoculated into index mice (**Extended Data Fig. 9b,c**), confirming that the *K.*

23   *pneumoniae* isolated from contact mice originated from the index animals. Secondly, we orally

24   inoculated the protective Bacteroidetes commensals into *K. pneumoniae* colonized neonatal index mice,

25   and also naïve neonatal contact mice, prior to cohousing. This was sufficient to block transmission of *K.*

26   *pneumoniae* between hosts (**Extended Data Figure 10, experiment 10 and Supplementary Fig. 10a**).

27   To examine whether transmission required direct contact of animals, adult contacts were housed in a

cage that had previously housed intestinally colonized index mice. Despite all index mice and fecal material being removed from the cage prior to housing, all contact mice became colonized by *K. pneumoniae* (**Extended Data Figure 10, experiment 11**), demonstrating that transmission does not require direct contact between animals or coprophagy. Thirdly, we orally inoculated the protective Bacteroidetes commensals into both *K. pneumoniae* colonized adult index mice, and naïve antibiotic treated adult contact mice, prior to cohousing. We found that Bacteroidetes specifically were sufficient to block transmission of *K. pneumoniae* between hosts (**Extended Data Figure 10, experiments 12-15, and Supplementary Fig. 10b**). The inhibitory effect of Bacteroidetes was lost upon disruption of IL-36 signaling or in the absence of the commensal colonization factors (**Extended Data Figure 10, experiments 16-18**). Taken together, these data suggest that the protective effect of Bacteroidetes on *K. pneumoniae* colonization limits its transmission within a population. We questioned whether the inhibitory effect of Bacteroidetes on *K. pneumoniae* transmission was due to Bacteroidetes reducing *K. pneumoniae* shedding from the index animals, or by establishing a protective barrier against *K. pneumoniae* acquisition in contact animals. To answer this, Bacteroidetes were given to either the *K. pneumoniae* colonized index mice only, or the naïve contacts only. We found that both approaches reduced *K. pneumoniae* transmission but that introduction of Bacteroidetes to contacts was more effective than the effect of Bacteroidetes in index mice (**Extended Data Figure 10, experiments 19 and 20**), demonstrating that the critical point in transmission controlled by Bacteroidetes is fortification of the immune barrier to prevent *K. pneumoniae* establishment in a new host. Collectively, our data show that CCF-producing Bacteroidetes inhibit the spread of *K. pneumoniae* within a population.

1 **DISCUSSION**

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3 Whilst it has been assumed that colonization resistance is a property common to resident microbial

4 communities colonizing different host mucosae, whether the rules and mechanisms of colonization

5 resistance for a given pathogen are shared between different niches is poorly defined[45]. We reveal that

6 microbiota development drives separate host defence programs in the adult intestine and upper airway

7 to inhibit colonization by the *K. pneumoniae* (**Supplementary Fig. 11**). This major antibiotic-resistant

8 pathogen can overcome these defences in the upper airway but cannot compete with the microbiota in

9 the intestine. Because of this, microbiota dysbiosis within an individual might not only promote *K.*

10 *pneumoniae* colonization in that host but also promotes its spread to others. Thus, the defined

11 consortium of Bacteroidetes we identify here could provide population level protection against *K.*

12 *pneumoniae* by preventing its transmission between hosts – the ultimate means of infectious disease

13 control.

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1 **COMPETING INTERESTS**

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3 The authors declare no competing interests.

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AUTHOR CONTRIBUTIONS

T.B.C. and R.P.S. conceived and performed all colonization, transmission and immunological experiments, analysed data, and wrote the manuscript. J.A.K.M. and J.R.M. performed sequencing experiments, analysed sequencing data and contributed to the manuscript. J.R.M. and T.B.C. acquired funding.

FIGURE LEGENDS

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3 **Figure 1. The adult microbiota protects against colonization by antibiotic-resistant *Klebsiella***

4 ***pneumoniae* in the intestine but not upper airway.** (**a**) Experimental scheme for (**b-j**): "ABX" =

5 metronidazole, neomycin, vancomycin and ampicillin (MNVA) in drinking water; "*Kp*" = *K. pneumoniae*

6 oral or intranasal inoculation; and "Sample" = upper airway or fecal sampling day. Days relative to *K.*

7 *pneumoniae* inoculation. (**b,c**) *K. pneumoniae* (OXA-48) burden in the (**b**) upper airway (n=8 animals)

8 or (**c**) colon of neonatal mice (n=8 animals). (**d-f**) *K. pneumoniae* (OXA-48) (n=8 animals) (**d**), *K.*

9 *pneumoniae* (B5055) (n=8,9 animals) (**e**), and *K. pneumoniae* (ST258) (n=7 animals) (**f**) burden in upper

10 airways of adult mice. (**g-j**) *K. pneumoniae* (OXA-48) (n=6 animals) (**g**), *K. pneumoniae* (B5055) (n=8

11 animals) (**h**), *K. pneumoniae* (ST258) (n=7 animals) (**i**) and *K. quasipneumoniae subsp.*

12 *similipneumoniae* (n=5 animals) (**j**) fecal burden in adult mice. (**k**) Experimental scheme for (**l,m**): "ABX"

13 = MNVA in drinking water; "MT" = microbiota transfer by oral and intranasal inoculation; "*Kp*" = *K.*

14 *pneumoniae* oral inoculation; and "Sample" = fecal sampling day. *K. pneumoniae* (OXA-48) (n=5

15 animals) (**l**) and *K. pneumoniae* (ST258) (n=6 animals) (**m**) fecal burden in adult mice. Upper airway

16 colonization data displayed in red and intestinal colonization data in blue. All statistical comparisons

17 were made by Mann-Whitney test (two-tailed), horizontal lines indicate median values, ND is none

18 detected (limit of detection for *K. pneumoniae* in feces = 10^3 CFU/g).

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20 **Figure 2. Intestinal Bacteroidetes protect against *Klebsiella pneumoniae* colonization.** (**a,b**)

21 Relative abundance of bacterial phyla (**a**) and orders (**b**) in (adult feces n=5 animals) and large intestine

22 of neonatal mice (n=6 animals). Each bar represents a single mouse. (**c**) Experimental scheme for (**d-**

23 **g**): "ABX" = MNVA in drinking water; "GF" = germ-free mice; "CC" = commensal consortia inoculation

24 by oral gavage (see **Materials and Methods**); "*Kp*" = *K. pneumoniae* oral inoculation; and "Sample" =

25 fecal sampling day. Days relative to *K. pneumoniae* inoculation. (**d-f**) *K. pneumoniae* (OXA-48) (n=7,8

26 animals) (**d**), *K. pneumoniae* (B5055) (n=8 animals) (**e**), and *K. pneumoniae* (ST258) (n=7,8 animals)

27 (**f**) fecal burden in adult antibiotic treated mice. (**g**) *K. pneumoniae* (ST258) fecal burden in germ-free

mice (n=5 animals). (**h**) Experimental scheme for (**i-k**): acronyms as in (**c**). *K. pneumoniae* (OXA-48) (n=5 animals) (**i**) and *K. pneumoniae* (ST258) (n=7 animals) (**j**) fecal burden in adult mice. *K. pneumoniae* (ST258) (**k**) fecal burden in neonatal mice (n=6,7 animals). All statistical comparisons were made by Kruskal-Wallis test with Dunn's correction for multiple comparisons. Horizontal lines indicate median values.

Figure 3. Bacteroidetes protect against *Klebsiella pneumoniae* colonization in the intestine through IL-36 signalling and macrophages. (**a**) Experimental scheme for (**b-e**): "ABX" = MNVA in drinking water; "CC" = commensal consortia oral inoculation; "*Kp*" = *K. pneumoniae* oral inoculation; and "Sample" = fecal sampling day; "Antibody treatment" = antibody, or isotype control, treatment. Days relative to *K. pneumoniae* inoculation. (**b-e**) *K. pneumoniae* (OXA-48) burden in the feces of adult mice (n=5,7,8 animals). (**e**) *K. pneumoniae* (ST258) burden in the feces of adult mice (n=6 animals). (**f**) Experimental scheme for (**g-i**): acronyms as in (**a**), days on which recombinant IL-36γ was administered by intraperitoneal injection are indicated. (**g,h**) *K. pneumoniae* (OXA-48) burden (n=6,7 animals) (**g**), and *K. pneumoniae* (ST258) burden (n=6 animals) (**h**) in the feces of adult mice. (**i**) Intestinal cytokine levels 3 days after oral inoculation with indicated commensal consortia (n=5 animals). (**j**) Experimental plan for (**k-n**), acronyms in experimental plan as in (**a**), "Clodronate" = day of clodronate liposome, or empty liposome treatment. (**k,l**) *K. pneumoniae* (OXA-48) burden (n=7,8 animals) (**k**), and *K. pneumoniae* (ST258) burden (n=5,6 animals) (**l**) in the feces of adult mice. (**m**) Intestinal cytokine levels 3 days after oral inoculation with indicated commensal consortia (n=5). (**n**) *K. pneumoniae* (OXA-48) fecal burden in adult mice (n=6,7 animals). Statistical comparisons were made by Kruskal–Wallis test with Dunn's correction for multiple comparisons (**b-e**, **i-l** and **n**), and by Mann-Whitney test (two-tailed) (**g**,**h**,**m**). Horizontal lines indicate median values, error bars are standard deviation.

Figure 4. Bacteroidetes require their commensal colonization factors to protect against *Klebsiella pneumoniae* colonization in the intestine. (**a**) Experimental scheme: "ABX" = MNVA in drinking water; "CC" = commensal consortia oral inoculation; "*Kp*" = *K. pneumoniae* oral inoculation;

1 and "Sample" = fecal sampling day. Days relative to *K. pneumoniae* inoculation. (**b**) *K. pneumoniae*

2 (OXA-48) fecal burden in adult mice (n=5 animals). Mice were orally inoculated with either a consortium

3 of wild-type or isogenic ΔCCF Bacteroidetes (*B. fragilis* and *B. vulgatus*). (**d**) Bacteroidetes levels in

4 feces of mice from (**b**). (**d**) Intestinal cytokine levels 3 days after oral inoculation of Bacteroidetes

5 consortia (n=5 animals). (**e,f**) *K. pneumoniae* (OXA-48) (n=6 animals) (**e**) and *K. pneumoniae* (ST258)

6 (n=6 animals) (**f**) fecal burden in adult mice. Indicated mice were orally inoculated with either WT *B.*

7 *fragilis*, ΔCCF *B. fragilis* or ΔCCF::CCF *B. fragilis.* (**g**) Intestinal cytokine levels 3 days after oral

8 inoculation with either WT *B. fragilis*, ΔCCF *B. fragilis* or ΔCCF::CCF *B. fragilis* (n=5). (**h**) Mucosal

9 association of WT *B. fragilis*, ΔCCF *B. fragilis* and ΔCCF::CCF *B. fragilis* (n=5,6 animals). (**i**) *B. fragilis*

10 levels in feces (n=5,6 animals). Statistical comparisons were made by Kruskal–Wallis test with Dunn's

11 correction for multiple comparisons (**b-c,e-i**), and by Student's *t*-test (two-tailed) (**d**). Horizontal lines

12 indicate median values, error bars are standard deviation.

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14 **Figure 5. Proteobacteria primes upper airway defences through IL-17A but encapsulation allows**

15 ***Klebsiella pneumoniae* to overcome these defences.** (**a**) Experimental scheme for (**b**): "ABX" =

16 MNVA in drinking water; "MT" = microbiota transfer; "*Kp*" = *K. pneumoniae* intranasal inoculation; and

17 "Sample" = upper airway lavage sampling; "Dexa" = dexamethasone treatment. Days relative to *K.*

18 *pneumoniae* inoculation. (**b**) Upper airway *K. pneumoniae* burden (WT B5055), unencapsulated *K.*

19 *pneumoniae* (ΔCPS B5055), and complemented *K. pneumoniae* (ΔCPS::CPS B5055) in adult mice

20 (n=7,9 animals). (**c**) Experimental plan for (**d-f**), acronyms as in (**a**), "Antibody treatment" = antibody, or

21 isotype control, treatment. Days relative to *K. pneumoniae* inoculation. (**d-f**) *K. pneumoniae* (WT and

22 ΔCPS B5055) upper airway burden in adult mice (n=5,7,8,9 animals). (**g**) Experimental scheme for (**h**):

23 acronyms as in (**a**), days of rIL-17A, or vehicle control, administration are indicated. (**h**) Upper airway

24 burden of unencapsulated *K. pneumoniae* (ΔCPS B5055), and complemented *K. pneumoniae*

25 (ΔCPS::CPS B5055) in adult mice (n=6 animals). (**i**) Experimental plan for (**j-m**), acronyms in

26 experimental plan as in (**a,c**), "CC" = indicated commensal consortia inoculation by intranasal inoculation

19

1 (see **Materials and Methods**). (**j-m**) Upper airway *K. pneumoniae* burden (WT B5055), unencapsulated

2 *K. pneumoniae* (ΔCPS B5055), and complemented *K. pneumoniae* (ΔCPS::CPS B5055) in adult mice

3 (n=5,7,8 animals). Mice were antibiotic treated in (**j**) and (**l**) and germ-free in (**k**). Statistical comparisons

4 were made by Kruskal–Wallis test with Dunn's correction for multiple comparisons (**b,f,h,j-m**) and by

5 Mann-Whitney test (two-tailed) (**d,e**). Horizontal lines are median values

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1 **MATERIALS AND METHODS**

2

3 **Bacterial Strains**. Bacteria used in this study and their growth conditions are outlined in

4 **Supplementary Table 1**.

5

6 **PCR amplification and sequencing of *wzi* gene.** To identify the capsule type of the *K. pneumoniae*

7 strains used in this study, genomic DNA was isolated from specified *K. pneumoniae* strains using the

8 Wizard Genomic DNA purification kit (Promega) as per the manufacturer's instructions. A 580 bp

9 segment of the *wzi* gene was amplified from the genomic DNA by PCR using the *wzi* primers outlined

10 in **Supplementary Table 2**[46]. The 25 μL PCR reaction contained 200 μM dNTPs, 0.5 μM of each primer,

11 5 μL genomic DNA template, 1 × GC buffer and 0.25 μL Phusion polymerase (New England Biolabs).

12 The PCR reaction was performed, following denaturation at 94°C for 2 minutes, by running 30 cycles

13 of: 94°C for 30 seconds; 55°C for 40 seconds; and 72°C for 30 seconds. A final elongation step of 72°C

14 for 5 minutes ended the reaction. The PCR was run on a 2% TAE agarose gel and the 580 bp band

15 excised and purified using the Monarch DNA gel extraction kit (New England Biolabs). Sanger

16 sequencing was performed on both strands using the PCR primers. The resulting consensus sequence

17 was used to identify its corresponding *wzi* gene using the capsular typing database

18 (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html).

19

20 **Mice**. The use of mice was performed under the authority of the UK Home Office outlined in the Animals

21 [Scientific Procedures] Act 1986 after ethical review by Imperial College London Animal Welfare and

22 Ethical Review Body (PPL 70/7969). Wild-type C57BL/6 mice were purchased from Charles River (UK).

23 Germ-free Swiss Webster mice were purchased from Taconic (Denmark). All adult mice were female

24 and between 6 − 14 weeks old, except the germ-free mice which were both male and female. Adult mice

25 were housed no more than five per cage with Aspen chip 2 bedding with a 12 hour light and 12 hour

1   dark cycle at $20^{o}C − 22^{o}C$. Neonatal mice were 7 – 11 days old. Mice were randomly assigned to

2   experimental groups, water was provided *ad libitum* and mice were fed RM1 (Special Diet Services).

3

4   **Microbiota-depletion, antibody, inhibitor and recombinant protein treatment.** Mice were given

5   broad-spectrum antibiotics (metronidazole 1 g/L, neomycin sulfate 1 g/L, ampicillin 1 g/L, and

6   vancomycin 0.5 g/L (MNVA)) in drinking water for 7 – 14 days as described previously[29]. For treatment

7   with a β-lactam and macrolide antibiotic combination, mice were given a total of four doses of

8   azithromycin (10 mg/kg/dose) and cefotaxime (40 mg/kg/dose) by intraperitoneal injection 1 and 2 days

9   prior to *K. pneumoniae* inoculation. Infant mice were indirectly exposed to antibiotics through their dams.

10   Dams were given the MNVA antibiotic cocktail in their drinking water as in[47]. Indicated groups of mice

11   were treated with dexamethasone (5 mg/kg/dose) (Sigma). Anti-IL-17A (R&D Systems), anti-IL-36R

12   (Invitrogen), anti-IFNγ (Abcam), and isotype control were administered at timepoints indicated in figures

13   prior to oral inoculation of *K. pneumoniae* via the intraperitoneal route at 75 μg/mouse to disrupt cytokine

14   signalling. Neutrophils were depleted using the anti-mouse Ly-6G antibody (1A8) (Biolegend).

15   Recombinant IL-17A (R&D Systems) and recombinant IL-36γ (R&D Systems) (10 μg/mouse) were

16   administered at timepoints indicated in figures prior to oral inoculation of *K. pneumoniae* with inoculation

17   via the intraperitoneal route. Macrophages were depleted using clodronate containing liposomes as

18   described previously[48].

19

20   **Models of bacterial colonization, quantification of bacterial load in the upper airway and**

21   **intestine.** For upper airway colonization of *K. pneumoniae* in adult mice, unanaesthetized animals were

22   intranasally administered indicated bacteria in 20 μL. In neonates, unanaesthetized animals were

23   intranasally administered *K. pneumoniae* in 5 μL. To establish intestinal colonization in adults,

24   unanaesthetized animals were orally inoculated with *K. pneumoniae* (5×10^5 CFU) in approximately 200

25   μL. For all experiments *K. pneumoniae* was grown to mid-log phase and resuspended in sterile PBS for

26   inoculation. Microbiota transfer was carried out as described in[29], 3 days prior to inoculation with *K.*

27   *pneumoniae*. Quantification of commensal bacterial load in the upper airway and intestine was carried

1   out essentially as described in[29]. For 16s rRNA analysis of consortia engraftment, we used primers

2   outlined in **Supplementary Table 2** (see **Microbiome sequencing**). To quantify *Klebsiella* in the upper

3   airway and intestine, *Klebsiella* ChromoSelect Selective Agar Base (Sigma) and for β-lactamase-

4   producing *Klebsiella*, ESBL ChromoSelect Agar Base (Sigma) were used as per the manufacturer's

5   instructions. We confirmed that the coloured colonies on these chromogenic plates were *Klebsiella* by

6   16s rRNA sequencing. For consortia inoculation mice were orally inoculated 5×10^8 CFU of either of the

7   following: the intestinal Bacteroidetes consortium consisted of: *B. fragilis, B. caccae, B.*

8   *thetaiotaomicron, B. uniformis, B.ovatus*; the intestinal Firmicutes consortium: *C. ramosum, C.*

9   *symbiosum, C. orbiscindens, C. histolyticum, E. faecalis;* the intestinal Actinobacteria consortium: *B.*

10 *adolescentis, E. lenta, B. breve*; and the intestinal Proteobacteria consortium: *E. coli, C. koseri, A.*

11 *radioresistens*. For all experiments using consortia of intestinal commensals, bacteria were grown to

12 mid-log phase. For anaerobic consortia (Firmicutes, Bacteroidetes, and Actinobacteria) bacteria were

13 resuspended in pre-reduced sterile PBS prior to inoculation, and for aerobic consortia (Proteobacteria)

14 bacteria were resuspended in sterile PBS prior to inoculation. For upper airway consortia inoculation

15 unanaesthetized animals mice were intranasally inoculated 1×10^6 CFU of either of the following in 20

16 μL: the upper airway Bacteroidetes consortium consisted of: *P. nigrescens* and *P. melaninogenica*; the

17 upper airway Firmicutes consortium: *S. epidermidis* and *L. crispatus*; the upper airway Actinobacteria

18 consortium: *P. acnes* and *C. propinquum*; and the upper airway Proteobacteria consortium: *H.*

19 *influenzae* and *M. catarrhalis*. For all experiments using consortia of upper airway commensals, bacteria

20 were grown to mid-log phase and consortia were resuspended in sterile PBS prior to inoculation.

21 Because of the small inoculation volume and lack of anesthesia these bacteria remain in the upper

22 airway[29].

23

24 **Cytokine measurements**. Cytokine levels were determined using ELISA kits for TNFα, IL-17A, IFNγ

25 (Biolegend) and IL-36γ (antibodies-online.com) according to the manufacturer's instructions.

26

Macrophage culture and killing assay. Authenticated J774A.1 macrophages (Sigma-Aldrich) were cultured in DMEM supplemented with 10% v/v FBS, penicillin (100 units/mL) and streptomycin (100 mg/mL) and maintained at $37^{\circ}C$ and 5% (v/v) CO_2. This cell line was free of mycoplasma contamination. For bone marrow-derived macrophages, bone marrow was harvested from the femurs and tibiae of C57BL/6 mice and differentiated into macrophages by culturing in R10 medium containing 15% L-cell conditioned media and maintained at $37^{\circ}C$ and 5% (v/v) CO_2. Macrophage killing assays were carried out essentially as described before[29,49].

Microbiome sequencing. For DNA extraction from feces, lung and upper airway lavage fluid DNA extracted using the E.Z.N.A Stool DNA kit (Omega Bio-Tek) as per the manufacturer's instructions. Homogenization of the stool and lung was accomplished by bead disruption with approximately 200 mg beads using a FastPrep-24 instrument (MP Biomedicals) by 15 repeated pulses of 20 seconds vortexing and a subsequent 2 minute recovery. All samples were vortexed for 30 seconds to ensure complete disruption before proceeding with the protocol. Processing included the optional extra steps of incubating at $95^{\circ}C$ to ensure sufficient lysis of Gram-positive bacteria, and treatment with 100 μg of RNase A for 3 minutes at $37^{\circ}C$ to remove unwanted RNA. For quantification of mucosa-associated bacteria, mucus was scraped from intestine that had been washed with sterile PBS to remove all fecal matter, as described in[31] and DNA extracted by bead beating as above. Extracted DNA was then used for 16s rRNA gene qPCR and deep sequencing. For qPCR, in a total 10 μL reaction there was 1 × SYBR Green PCR Master Mix (Thermo Fisher Scientific), 200 nM of each forward and reverse primer (**Supplementary Table 2**), and 2 μL template DNA. qPCR was performed on an Applied Biosystems StepOnePlus instrument and cycled 40 times with 15 seconds at 95 °C and 1 min at $61^{\circ}C$. To calculate 16s rRNA gene copy numbers, a standard curve was generated using plasmids containing a cloned 16s rRNA gene from a representative bacterial species of the indicated phylum (**Supplementary Table 2**). Plasmid copy numbers were calculated using the formula: (amount of plasmid in ng $\times 6.0221 \times 10^{23}$ molecules/mol)/((4500 × 660 g/mol) × 1 × 10^9 ng/g), where 4500 is the estimated length of the plasmid with the 16s rRNA gene insert and 660 g/mol is the average mass of 1 base of dsDNA. Using this

1 standard curve, 16s rRNA gene copy number per gram of stool was determined. 16s rRNA gene

2 sequencing sample libraries were generated using Illumina's 16s Metagenomic Sequencing Library

3 Preparation Protocol with some modifications. The V1-V2 16s rRNA gene regions were amplified from

4 stool isolated DNA using primers at a ratio as described previously [50]. Index PCR reactions were cleaned

5 and subsequently normalized with the SequalPrep Normalization Plate Kit (Life Technologies). These

6 libraries were quantified by the NEBNext Library Quant Kit for Illumina (New England Biolabs).

7 Sequencing data was generated using the MiSeq Reagent Kit v3 (Illumina) and paired-end 300 bp

8 chemistry on an Illumina MiSeq platform (Illumina Inc). Sequencing data was processed following the

9 DADA2 pipeline as previously described[51]. The SILVA bacterial database version 132 was used to

10 classify the sequence variants (www.arb-silva.de/).

11

12 **Models of bacterial transmission**. For transmission studies in adult mice, "index" mice colonized by

13 *K. pneumoniae* either in the upper airway or intestine were cohoused with naïve "contact" mice at

14 indicated "index":"contact" ratios. "Contact" mice were either treated with azithromycin and cefotaxime

15 as above, or non-antibiotic treated as indicated. To study *K. pneumoniae* transmission in infant mice, a

16 single infant "index" mouse was colonized by *K. pneumoniae* in the intestine and returned to its litter of

17 naïve "contact" littermates. To determine whether direct contact between animals was required for

18 transmission, indicated naïve contact mice were housed in a cage which had previously housed *K.*

19 *pneumoniae* colonized "index" mice. Before the addition of the "contact" animals, all feces was removed

20 from the cage.

21

22 **Statistical and reproducibility.** Statistical comparisons were performed using GraphPad Prism 8

23 software. To compare differences between two groups, the Student's *t*-test or the Mann–Whitney test

24 were used as appropriate (two-tailed). For statistical analysis of transmission, Fisher's exact test was

25 used (two-sided). For multiple comparisons, a Kruskal–Wallis test with Dunn's correction for multiple

26 comparisons was used. All error bars indicated standard deviation. All experiments were from a

27 minimum of 4 biological replicates, with data from all replicates included in statistical analysis and no

1   data excluded. Animals were randomly assigned to groups (cages) prior to any experimentation.

2   Differences between litters of mouse pups were controlled for by mixing pups from different litters into

3   each treatment condition. We used power analysis from published studies and our preliminary

4   experiments to determine the minimum required sample size required for the groups used in our

5   experiments. Microbiota sample preparation and analysis performed in a blinded manner.

6

7   **Data availability.** Microbiome sequencing data have been deposited at the NCBI SRA (BioProject

8   PRJNA579139). The data supporting the findings of the study are available in this article (see Source

9   Data) and its Supplementary Information files, or from the corresponding author upon request.

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Figure 1.

a

Relative abundance (%)

Neonatal | Adult

Legend:
- Actinobacteria (purple)
- Bacteroidetes (blue)
- Firmicutes (red)
- Proteobacteria (green)
- Other Phyla (dark green)

b

Relative abundance (%)

Neonatal | Adult

Actinobacteria
- Coriobacteriales
- Micrococcales

Bacteroidetes
- Bacteroidales

Firmicutes
- Erysipelotrichales
- Clostridiales
- Lactobacillales
- Bacillales

Proteobacteria
- Rhodospirillales
- Betaproteobacteriales
- Pseudomonadales
- Enterobacteriales
- Desulfovibrionales

Other phyla
- Other Orders

c

ABX/GF | *Kp* | CC | CC

Day: -14   0   1   2   3

Sample

d

P>0.9999
P>0.9999
P>0.9999
P=0.0008

CFU/g stool

Bacteroidetes:	–	+	–	–	–
Firmicutes:	–	–	+	–	–
Actinobacteria:	–	–	–	+	–
Proteobacteria:	–	–	–	–	+

e

P>0.9999
P>0.9999
P=0.0065

CFU/g stool

Bacteroidetes:	–	+	–	–
Firmicutes:	–	–	+	–
Actinobacteria:	–	–	–	+

f

P=0.6084
P=0.3454
P=0.0220

CFU/g stool

Bacteroidetes:	–	+	–	–
Firmicutes:	–	–	+	–
Actinobacteria:	–	–	–	+

g

P>0.9999
P=3264
P=0.0019

CFU/g stool

Bacteroidetes:	–	+	–	–
Firmicutes:	–	–	+	–
Actinobacteria:	–	–	–	+

h

ABX | CC | CC | *Kp*

Day: -14   0   1   2   3

Sample

i

P>0.9999
P=0.7183
P=0.0040

CFU/g stool

Bacteroidetes:	–	+	–	–
Firmicutes:	–	–	+	–
Actinobacteria:	–	–	–	+

j

P=0.3922
P=0.4724
P<0.0001

CFU/g stool

Bacteroidetes:	–	+	–	–
Firmicutes:	–	–	+	–
Actinobacteria:	–	–	–	+

k

P>0.9999
P=0.0042

CFU/g stool

| Bacteroidetes: | – | + | – |
| Firmicutes: | – | – | + |

Figure 2.

Figure 3.

a

ABX | CC | CC | *Kp*

Day: -14   0   1   2   3

Sample

b

P>0.9999

P=0.0112   *P*=0.0486

CFU/g stool

10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}

Bacteroidetes: –   WT   ΔCCF
consortium

c

P=0.0155

P=0.0360   *P*>0.9999

Bacteroidetes 16s rRNA
gene copies
(per g of feces)

10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}

Bacteroidetes: –   WT   ΔCCF
consortium

d

P<0.0001

IL-36γ (pg/mL)

150
100
50

Bacteroidetes: WT   ΔCCF
consortium

e

P=0.0303

P>0.9999

P=0.0038

CFU/g stool

10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}

B. fragilis: –   WT   ΔCCF   ΔCCF
::CCF

f

P>0.9999

P>0.9999

P=0.0295   *P*=0.0096

CFU/g stool

10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}

B. fragilis: –   WT   ΔCCF   ΔCCF
::CCF

g

P=0.1251

P>0.0001

IL-36γ (pg/mL)

200
150
100
50

B. fragilis: WT   ΔCCF   ΔCCF
::CCF

h

P=0.8978

P<0.0001

Mucosal colonization by *B. fragilis*
(% relative to mucosally
associated WT *B. fragilis*
per cm of intestine)

150
100
50

B. fragilis: WT   ΔCCF   ΔCCF
::CCF

i

P>0.9999

P>0.9999

B. fragilis 16s rRNA
gene copies
(per g of feces)

10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}

B. fragilis: WT   ΔCCF   ΔCCF
::CCF

Figure 4.

Figure 5.
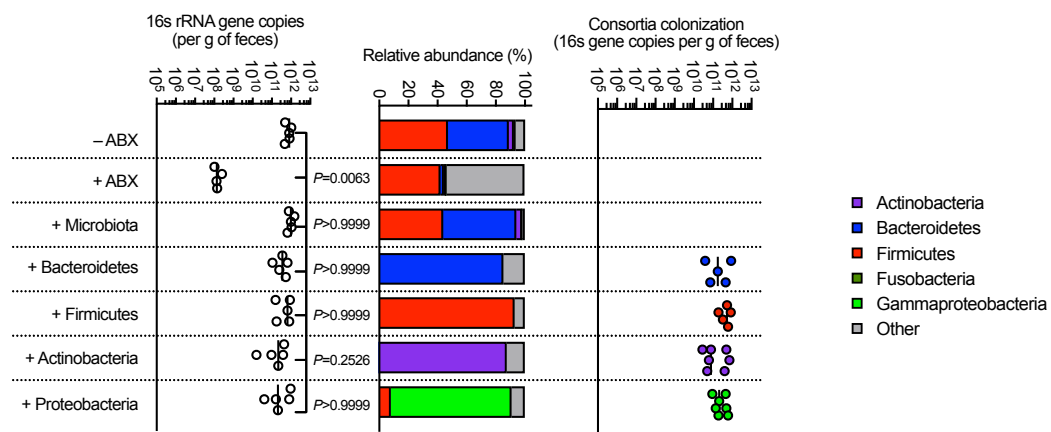
Firmicutes: + −
Bacteroidetes: − +

Extended Data Figure 1

Extended Data Figure 2.

a

ABX | MT | *Kp*

Day: -17 ... -3 -1 0 2 4
Dexa:
Sample

b

CFU/g stool

P=0.025   *P*=0.0003

10^11
10^10
10^9
10^8
10^7
10^6
10^5
10^4
10^3

	Day 2		Day 4	
Adult microbiota:	+	+	+	+
Vehicle:	−	+	−	+
Dexamethasone:	+	−	+	−

c

Kp

Day: -3 -1 0 2
Dexa:
Sample

d

CFU/g stool

P=0.0003

10^11
10^10
10^9
10^8
10^7
10^6
10^5
10^4
10^3

ND

Vehicle:	+	−
Dexamethasone:	−	+

e

CFU/g stool

P=0.0006

10^11
10^10
10^9
10^8
10^7
10^6
10^5
10^4
10^3

ND

Vehicle:	+	−
Dexamethasone:	−	+

Intestine
● − ABX
○ + ABX

f

ABX | CC | CC | *Kp*

Day: -17 ... -3 -1 0 2
Dexa:
Sample

g

CFU/g stool

P=0.0006

10^11
10^10
10^9
10^8
10^7
10^6
10^5
10^4
10^3

Bacteroidetes:	+	+
Vehicle:	+	−
Dexamethasone:	−	+

Extended Data Figure 3.

a

Bacterial viability (%)

P=0.0022

100
80
60
40
20
0

Vehicle: + −
rIL-36γ: − +

b

Bacterial viability (%)

P=0.0101

100
80
60
40
20
0

Vehicle: + −
rIL-36γ: − +

c

Bacterial viability (%)

P=0.0239

80
60
40
20
0

Vehicle: + −
rIL-36γ: − +

d

Bacterial viability (%)

P=0.0359

150
100
50
0

Vehicle: + −
rIL-36γ: − +

Extended Data Figure 4.

a

Day: -17 -3 -1 0 1

ABX | MT | *Kp*

Dexa: ↑ ↑

Sample

b

P=0.3787
P<0.0001
P=0.3737
P=0.0003
P=0.0001

TNF (pg/mL)

Kp strain:	WT	WT	ΔCPS	ΔCPS ::CPS	ΔCPS	ΔCPS	ΔCPS
Adult microbiota:	–	–	–	–	–	+	+
Vehicle:	–	–	–	–	–	+	–
Dexamethasone:	–	–	–	–	–	–	+

c

P=0.0196
P>0.9999

16s rRNA gene copies
(per mL of URT lavage)

Adult microbiota:	+	+	–
Vehicle:	+	–	–
Dexamethasone:	–	+	–

Upper airway

● – ABX
○ + ABX

Extended Data Figure 5.

a

16s rRNA gene copies (per lung)

Relative abundance (%)

Legend:
- Actinobacteria (purple)
- Bacteroidetes (blue)
- Firmicutes (red)
- Fusobacteria (dark green)
- Gammaproteobacteria (green)
- Other (gray)

− ABX	
+ ABX	*P*<0.0001
+ Microbiota	*P*=0.9078
+ Bacteroidetes	*P*=0.9195
+ Firmicutes	*P*=0.4959
+ Actinobacteria	*P*=0.6767
+ Proteobacteria	*P*=0.9914

Extended Data Figure 6.

a

P>0.9999
P=0.8258
P>0.9999   *P*=0.3780

CFU/mouse

10⁵
10⁴
10³
10²
10¹

Kp strain: WT WT ΔCPS ΔCPS

b

P=0.9399
P=0.9394
P=0.4480   *P*=0.7991

TNF (pg/mL)

200
150
100
50
0

Kp strain: WT WT ΔCPS ΔCPS

Upper airway

● – ABX
○ + ABX

Extended Data Figure 7.

a

Day: -14 ... -3 ... -1 ... 0 ... 1

ABX | CC | CC | *Kp*

Sample

b

P=0.0011
P=0.9231
P=0.0016

TNF (pg/mL)

800
600
400
200
0

Kp strain:	ΔCPS	ΔCPS	ΔCPS	ΔCPS
Proteobacteria:	–	–	+	–
Firmicutes:	–	–	–	+

c

P=0.0175 P=0.0089

IL-17A (pg/mL)

1000
800
600
400
200
0

Proteobacteria:	–	–	+	–
Bacteroidetes:	–	–	–	+

Upper airway

● – ABX
○ + ABX

Extended Data Figure 8.

Extended Data Figure 9.

| Experiment	Index mice			Contact mice			Transmission % (number of index mice:contact mice)	Statistical significance
	Age	*Klebsiella* Reservoir	Microbiota /immune manipulation	Age	Mucosa of *Klebsiella* acquisition	Microbiota /immune manipulation		
1	Neonates	Airway	–	Neonates	Airway	–	0% (2:8)	–
2	Neonates	Airway	–	Neonates	Airway	+ Antibiotics	0% (2:8)	–
3	Neonates	Airway	–	Neonates	Intestine		12.5% (2:8)	–
4	Adult	Airway	–	Adult	Airway	–	0% (8:12)	–
5	Neonates	Intestine	–	Neonates	Intestine	–	100% (2:12)	–
6	Adult	Intestine	+ Antibiotics	Adult	Intestine	+ Antibiotics	100% (3:12)	–
7	Adult	Intestine	+ Antibiotics	Adult	Intestine	+ Antibiotics	100% (2:8)	–
8	Adult	Intestine	+ Antibiotics	Adult	Intestine	–	0% (3:12)	*P*<0.0001 (Exp 6 vs Exp 8)
9	Adult	Intestine	+ Antibiotics	Adult	Intestine	–	8.3% (3:12)	*P*<0.0001 (Exp 7 vs Exp 9)
10	Neonates	Intestine	+ Bacteroidetes	Neonates	Intestine	+ Bacteroidetes	0% (3:12)	*P*<0.0001 (Exp 5 vs Exp10)
11	Adult	Intestine	+ Antibiotics	Adult	Intestine	+ Antibiotics	100% (indirect exposure) (2:8)	–
12	Adult	Intestine	+ Antibiotics + Bacteroidetes (WT)	Adult	Intestine	+ Antibiotics + Bacteroidetes (WT)	0% (3:12)	–
13	Adult	Intestine	+ Antibiotics + Firmicutes	Adult	Intestine	+ Antibiotics + Firmicutes	100% (3:12)	–
14	Adult	Intestine	+ Antibiotics + Bacteroidetes (WT)	Adult	Intestine	+ Antibiotics + Bacteroidetes (WT)	0% (3:12)	–
15	Adult	Intestine	+ Antibiotics + Firmicutes	Adult	Intestine	+ Antibiotics + Firmicutes	100% (3:12)	–
16	Adult	Intestine	+ Antibiotics + Bacteroidetes (ΔCCF)	Adult	Intestine	+ Antibiotics + Bacteroidetes (ΔCCF)	100% (3:12)	*P*<0.0001 (Exp 12 vs Exp16)
17	Adult	Intestine	+ Antibiotics + Bacteroidetes (WT) +αIL-36R	Adult	Intestine	+ Antibiotics + Bacteroidetes (WT) +αIL-36R	100% (3:12)	*P*<0.0001 (Exp 12 vs Exp 17)
18	Adult	Intestine	+ Antibiotics + Bacteroidetes (WT) +αIL-36R	Adult	Intestine	+ Antibiotics + Bacteroidetes (WT) +αIL-36R	100% (3:12)	*P*<0.0001 (Exp 14 vs Exp 18)
19	Adult	Intestine	+ Antibiotics	Adult	Intestine	+ Antibiotics + Bacteroidetes (WT)	5% (5:20)	–
20	Adult	Intestine	+ Antibiotics + Bacteroidetes (WT)	Adult	Intestine	+ Antibiotics	35% (5:20)	*P*=0.0436 (Exp 19 vs Exp 20)

Extended Data Figure 10.
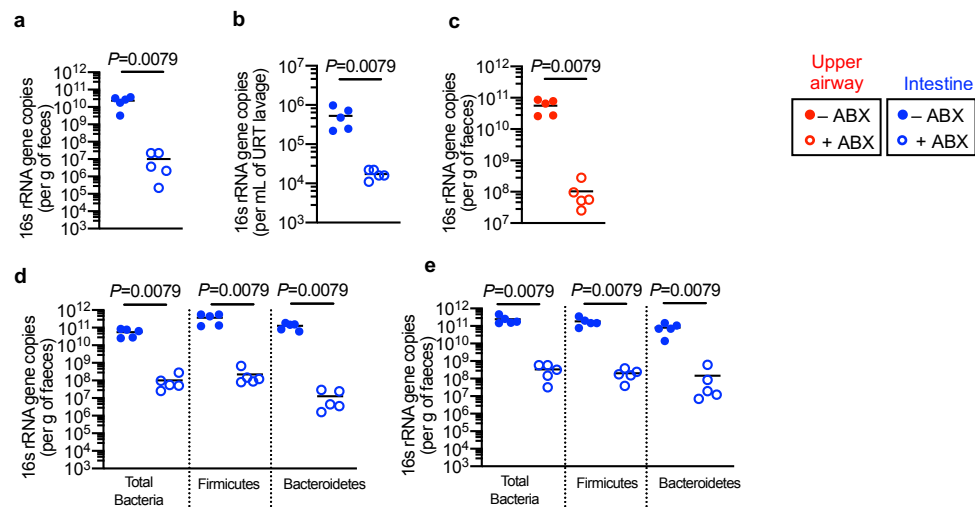
Supplementary Figure 1. Supplementary Figure 1. Antibiotics deplete the microbiota in neonatal and adult mice. (**a,b**) Levels of commensal bacteria the feces (n=5 animals) and upper airway (n=5 animals) of neonatal mice measured by total 16s rRNA gene copies. (**c**) Levels of commensal bacteria in the upper airway of adult mice (n=5 animals) measured by total 16s rRNA gene copies. (**d,e**) Levels of commensal bacteria in feces of adult mice (n=5 animals) measured by 16s rRNA gene copies (total bacteria, Firmicutes, and Bacteroidetes). Antibiotics were MNVA (**a-d**) and azithromycin and cefotaxime (**e**). Statistical comparisons were by Mann-Whitney (two-tailed), horizontal lines indicate median values.
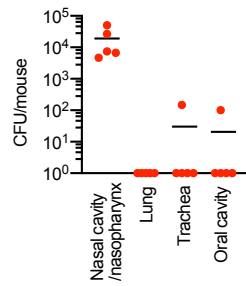
Supplementary Figure 2. . Validation of upper airway colonization by *K. pneumoniae*.
Burden of *K. pneumoniae* (WT B5055) in adult mice at indicated sites 1 day post-intranasal
inoculation (n=5 animals). Mice were intranasally inoculated 5×10^5 CFU. Horizontal lines
indicate median values.
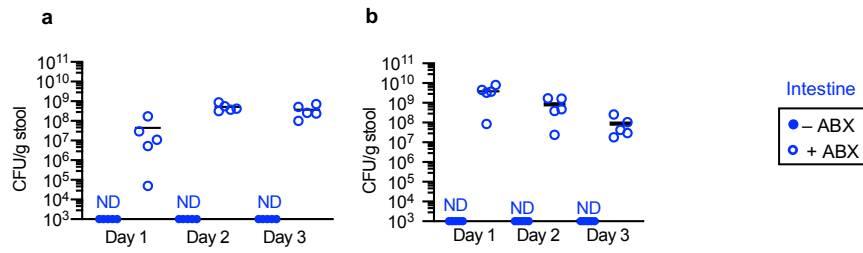
Supplementary Figure 3. . Clinically relevant antibiotic regimens promote intestinal colonization by _K. pneumoniae_. (**a,b**) _K. pneumoniae_ (OXA-48) (**a**), _K. pneumoniae_ (B5055) and (**b**) fecal burden in adult mice. Antibiotics were azithromycin and cefotaxime. Days indicated are post-inoculation with _K. pneumoniae_, ND is none detected (the limit of detection for _K. pneumoniae_ in feces = 10^3 CFU/g) and horizontal lines indicate median values.
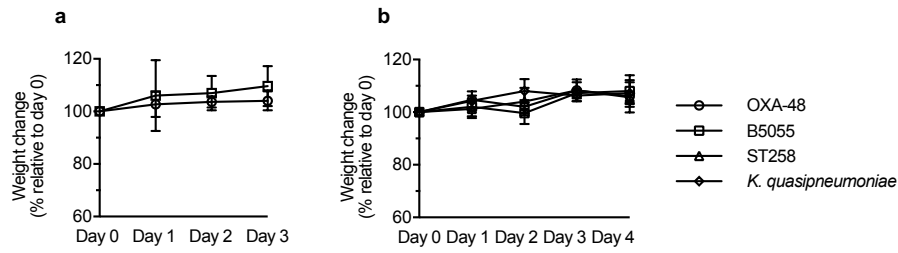
Supplementary Figure 4. Colonization of the upper airway or intestine does not promote weight loss. (**a,b**) Change in weight of mice colonized by indicated Klebsiella (K. pneumoniae OXA-48, B5055, ST258 or *K. quasipneumoniae subsp. similipneumoniae*) in the upper airway (A) and intestine (B) (n=7 animals). Indicated days are post-*K. pneumoniae* inoculation, error bars are standard deviation.
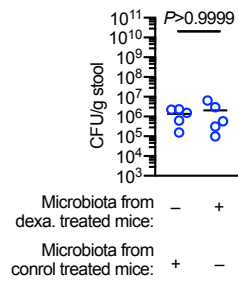
Supplementary Figure 5. Dexamethasone treatment does not cause changes to the microbiota preventing it protecting against *K. pneumoniae*. *K. pneumoniae* (OXA-48) burden in the feces of adult 3 days post-oral inoculation of *K. pneumoniae* (n=5 animals). Microbiota transfer was 3 days prior to oral inoculation of *K. pneumoniae* (5×10^5 CFU). Statistical comparison was by Mann-Whitney (two-tailed), horizontal lines indicate mean values.
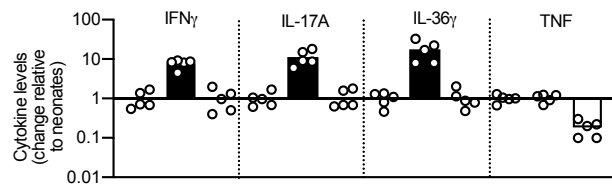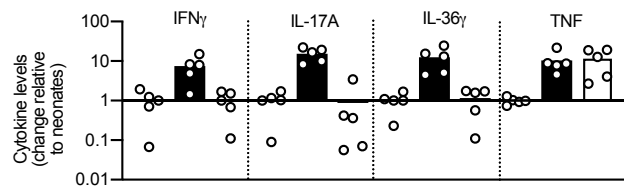
Supplementary Figure 6. The transition from neonate to adult is accompanied by changes in intestinal cytokine production. Change in cytokine level measured by ELISA in the intestine from neonate to adult. For each cytokine, from left to right the samples are: neonatal (non-antibiotic treated), adult (non-antibiotic treated), and adult (antibiotic treated). Antibiotics were MNVA (n=5 animals). Bars represent mean values.
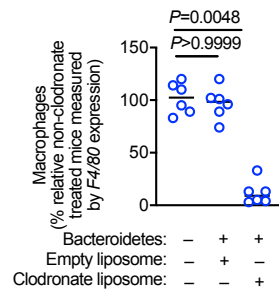
Supplementary Figure 7. Clodronate depletes macrophages in the intestine. The expression of the macrophage marker F4/80 was measured as in[27-30] in the intestinal tissue of antibiotic treated mice, antibiotic treated mice administered the Bacteroidetes consortium (as in **Fig. 2**) and clodronate liposomes, and antibiotic treated mice administered the Bacteroidetes consortium and empty liposomes (n=5 animals). Statistical comparisons were by Kruskal–Wallis test with Dunn's correction for multiple comparisons, horizontal lines indicate median values.
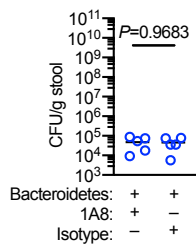
Supplementary Figure 8. Neutrophils are not required for Bacteroidetes to protect against *K. pneumoniae* in the intestine. *K. pneumoniae* (OXA-48) burden in the feces of adult mice 2 days post-inoculation (n=5 animals). Bacteroidetes administered as in **Fig. 2**. Antibodies administered 3 and 1 days before Bacteroidetes (100 μg/mouse via intraperitoneal injection). Statistical comparison was by Mann-Whitney (two-tailed), horizontal lines indicate mean values.

Supplementary Figure 9. The transition from neonate to adult is accompanied by changes in upper airway cytokine production. Change in cytokine level in the upper airway measured by ELISA from neonate to adult. For each cytokine, from left to right the samples are: neonatal (non-antibiotic treated), adult (non-antibiotic treated), and adult (antibiotic treated). Antibiotics were MNVA (n=5 animals). Bars represent mean values.
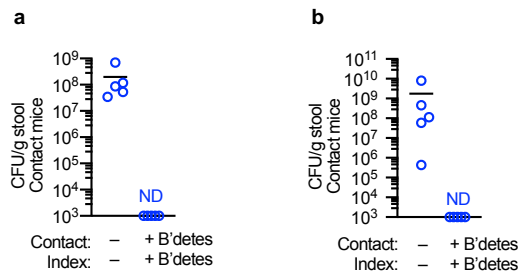
Supplementary Figure 10. Bacteroidetes protect against K. pneumoniae transmission in neonatal and adult mice. (**a,b**) *K. pneumoniae* (OXA-48) fecal burden in neonatal (**a**) and adult (**b**) contact mice after 4 days cohousing (n=5 animals). Indicated groups were administered the intestinal Bacteroidetes consortia as in **Fig. 2.** ND is none detected (the limit of detection for *K. pneumoniae* in feces = 10^3 CFU/g) and horizontal lines indicate median values.
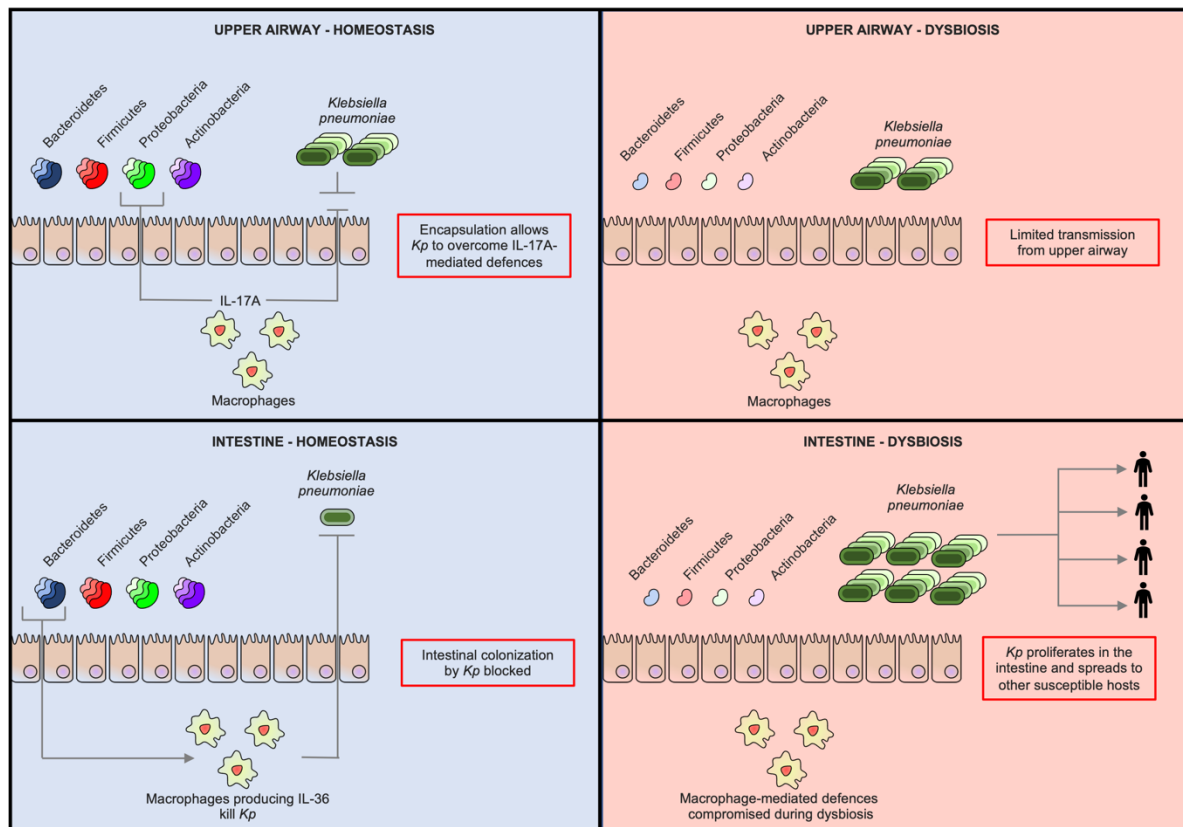
Supplementary Figure 11. Proposed model for microbiota programmed immune defences against *Klebsiella pneumoniae* colonization in the upper airway and intestine. The maturation of the adult microbiota drives the development of immune defences in the upper airway and intestine. In the upper airway Proteobacteria enhance IL-17A mediated defences against *Klebsiella pneumoniae*, whereas in the intestine Bacteroidetes enhance IL-36 mediated defences against *Klebsiella pneumoniae*. In the upper airway *Klebsiella pneumoniae* overcomes IL-17A-mediated defences through encapsulation to successfully colonize this site. By contrast, *Klebsiella pneumoniae* is unable to withstand the macrophage driven immune defences in the intestine and is block from colonization of this niche. Dysbiosis of the microbiota facilitates intestinal colonization by *Klebsiella pneumoniae* and this is the reservoir of transmission between hosts. Thus, the protection provided by intestinal Bacteroidetes not only protects an individual host against *Klebsiella pneumoniae* it also protects the whole population by preventing it's spread between hosts.

Bacterium	Phylum	Growth media	Growth conditions	*wzi* sequence type	Associated Capsule type
Bacteroides fragilis (WT)	Bacteroidetes	Brain heart infusion + 5% (v/v) defibrinated Sheep's blood	Anaerobic, 37ºC	–	–
Bacteroides fragilis (∆CCF)	Bacteroidetes	Brain heart infusion + 5% (v/v) defibrinated Sheep's blood	Anaerobic, 37ºC	–	–
Bacteroides fragilis (∆CCF::CCF) (complemented with the *B. fragilis* CCF operon)	Bacteroidetes	Brain heart infusion + 5% (v/v) defibrinated Sheep's blood + chloramphenicol	Anaerobic, 37ºC	–	–
Bacteroides vulgatus (WT)	Bacteroidetes	Brain heart infusion + 5% (v/v) defibrinated Sheep's blood	Anaerobic, 37ºC	–	–
Bacteroides vulgatus(∆CCF)	Bacteroidetes	Brain heart infusion + 5% (v/v) defibrinated Sheep's blood	Anaerobic, 37ºC	–	–
Bacteroides caccae	Bacteroidetes	Supplemented brain heart infusion	Anaerobic, 37ºC	–	–
Bacteroides thetaiotaomicron	Bacteroidetes	Brain heart infusion + 5% (v/v) defibrinated Sheep's blood	Anaerobic, 37ºC	–	–
Bacteroides uniformis	Bacteroidetes	Brain heart infusion + 5% (v/v) defibrinated Sheep's blood	Anaerobic, 37ºC	–	–
Bacteroides ovatus	Bacteroidetes	Supplemented brain heart infusion	Anaerobic, 37ºC	–	–
Bacteroides dorei	Bacteroidetes	Clostridial differential broth	Anaerobic, 37ºC	–	–
Bacteroides finegoldi	Bacteroidetes	Liver broth	Anaerobic, 37ºC	–	–
Prevotella nigrescens	Bacteroidetes	Tryptic soy broth + 5% (v/v) defibrinated	Anaerobic, 37ºC	–	–

		Sheep's blood			
Prevotella melaninogenica	Bacteroidetes	Chopped meat media	Anaerobic, 37°C	–	–
Clostridium ramosum	Firmicutes	Brain heart infusion	Anaerobic, 37°C	–	–
Clostridium symbiosum	Firmicutes	Tryptic soy broth + 5% (v/v) defibrinated Sheep's blood	Anaerobic, 37°C	–	–
Clostridium orbiscindens	Firmicutes	Chopped meat media	Anaerobic, 37°C	–	–
Clostridium histolyticum	Firmicutes	Tryptic soy broth + 5% (v/v) defibrinated Sheep's blood	Anaerobic, 37°C	–	–
Enterococcus faecalis	Firmicutes	Brain heart infusion	Anaerobic, 37°C	–	–
Lactobacillus reuteri	Firmicutes	DeMan, Rogosa and Sharpe broth	Aerobic, 37°C	–	–
Lactobacillus johnsonii	Firmicutes	DeMan, Rogosa and Sharpe broth	Aerobic, 37°C	–	–
Staphylococcus epidermidis	Firmicutes	Brain heart infusion	Aerobic, 37°C	–	–
Lactobacillus crispatus	Firmicutes	DeMan, Rogosa and Sharpe broth	Aerobic, 37°C	–	–
Bifidobacterium adolescentis	Actinobacteria	Reinforced Clostridial broth	Anaerobic, 37°C	–	–
Eggerthella lenta	Actinobacteria	Chopped meat media	Anaerobic, 37°C	–	–
Bifidobacterium breve	Actinobacteria	Tryptic soy broth + 5% (v/v) defibrinated Sheep's blood	Anaerobic, 37°C	–	–
Propionibacterium acnes	Actinobacteria	Brain heart infusion	Aerobic, 37°C	–	–
Corynebacterium propinquum	Actinobacteria	Brain heart infusion	Aerobic, 37°C	–	–
Escherichia coli	Proteobacteria	Luria-Bertani (Lennox) broth	Aerobic, 37°C	–	–
Citrobacter koseri	Proteobacteria	Brain heart infusion	Aerobic, 37°C	–	–
Acinetobacter radioresistens	Proteobacteria	Tryptic soy broth	Aerobic, 37°C	–	–
Haemophilus influenzae	Proteobacteria	Supplemented brain heart infusion	Aerobic, 37°C	–	–
Moraxella catarrhalis	Proteobacteria	Brain heart infusion	Aerobic, 37°C	–	–

K. pneumoniae (OXA-48) ABX resistance: ampicillin (50 mg/mL), azithromycin (50 mg/mL). ABX sensitivity: kanamycin (50 mg/mL)	Proteobacteria	Luria-Bertani (Lennox) broth	Aerobic, 37°C	24	K24
K. pneumoniae (ST258) ABX resistance: ampicillin (50 mg/mL), kanamycin (50 mg/mL). ABX sensitivity: gentamycin (50 mg/mL)	Proteobacteria	Luria-Bertani (Lennox) broth	Aerobic, 37°C	154	–
K. quasipneumoniae subsp. similipneumoniae	Proteobacteria	Luria-Bertani (Lennox) broth	Aerobic, 37°C	171	–
K. pneumoniae (WT B5055)	Proteobacteria	Luria-Bertani (Lennox) broth	Aerobic, 37°C	4	K2
K. pneumoniae (ΔCPS B5055)	Proteobacteria	Luria-Bertani (Lennox) broth	Aerobic, 37°C	–	–
K. pneumoniae (ΔCPS::CPS B5055) (complemented with pGEMT containing *wza-wzc*)	Proteobacteria	Luria-Bertani (Lennox) broth +ampicillin	Aerobic, 37°C	–	–

Supplementary Table 1. Bacteria used in this study.

Primer	Sequence (5' to 3')	Species used as a template to generate standard curve
16s F qRT	ACTCCTACGGGAGGCAGCAGT	*Clostridium symbiosum*
16s R qRT	ATTACCGCGGCTGCTGGC	
Firmicutes F	GGAGYATGTGGTTTAATTCGAAGCA	*Clostridium symbiosum*
Firmicutes R	AGCTGACGACAACCATGCAC	
Bacteroidetes F	GGARCATGTGGTTTAATTCGATGAT	*Bacteroides uniformis*
Bacteroidetes R	AGCTGACGACAACCATGCAG	
Actinobacteria F	TACGGCCGCAAGGCTA	*Micrococcus luteus*
Actinobacteria R	TCRTCCCCACCTTCCTCCG	
Fusobacteria F	AAGCGCGTCTAGGTGGTTATGT	*Fusobacterium necrophorum*
Fusobacteria R	TGTAGTTCCGCTTACCTCTCCAG	
Gammaproteobacteria F	TCGTCAGCTCGTGTYGTGA	*E. coli*
Gammaproteobacteria F	CGTAAGGGCCATGATG	
B. fragilis F	TGATTCCGCATGGTTTCATT	*B. fragilis*
B. fragilis R	CGACCCATAGAGCCTTCATC	
wzi F	GTGCCGCGAGCGCTTTCTATCTTGGTATTCC	N/A
wzi R	GAGAGCCACTGGTTCCAGAATTTCACCGC	N/A

Supplementary Table 2. Primers used in this study.