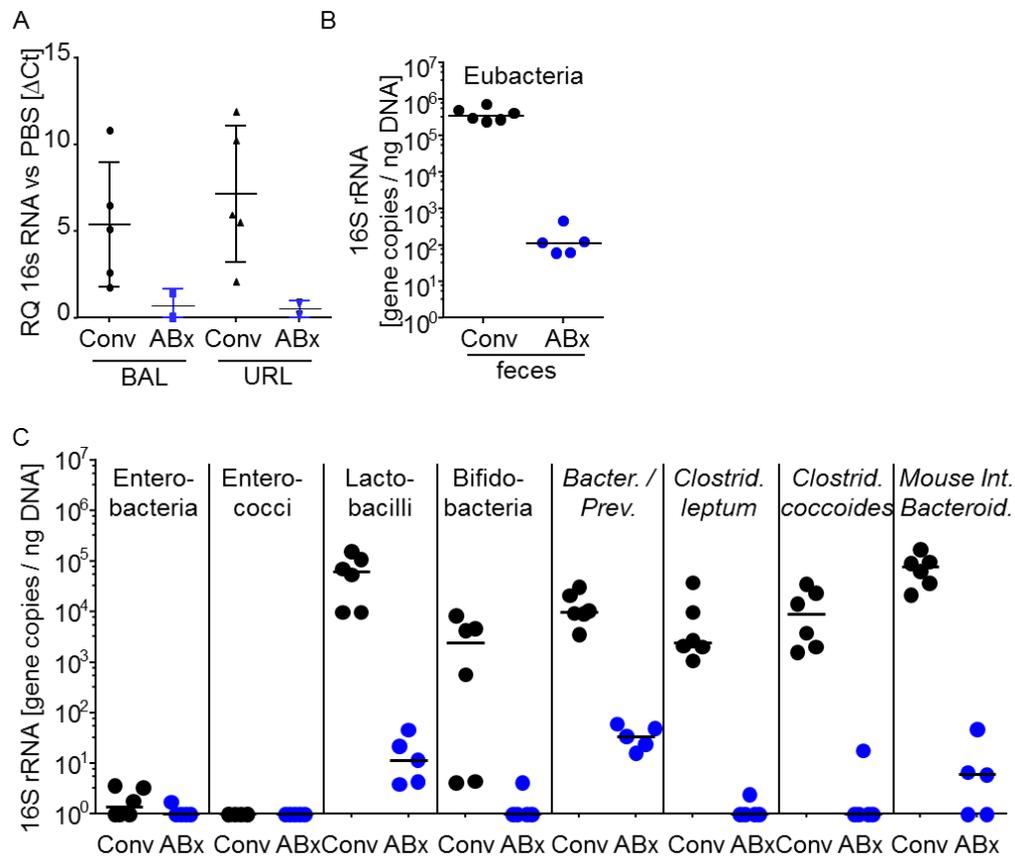
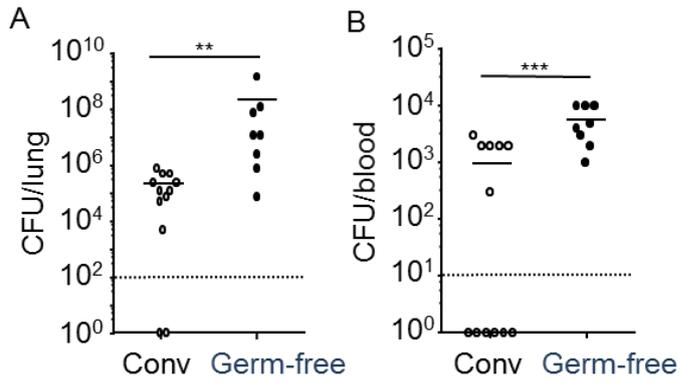


Supplemental data:

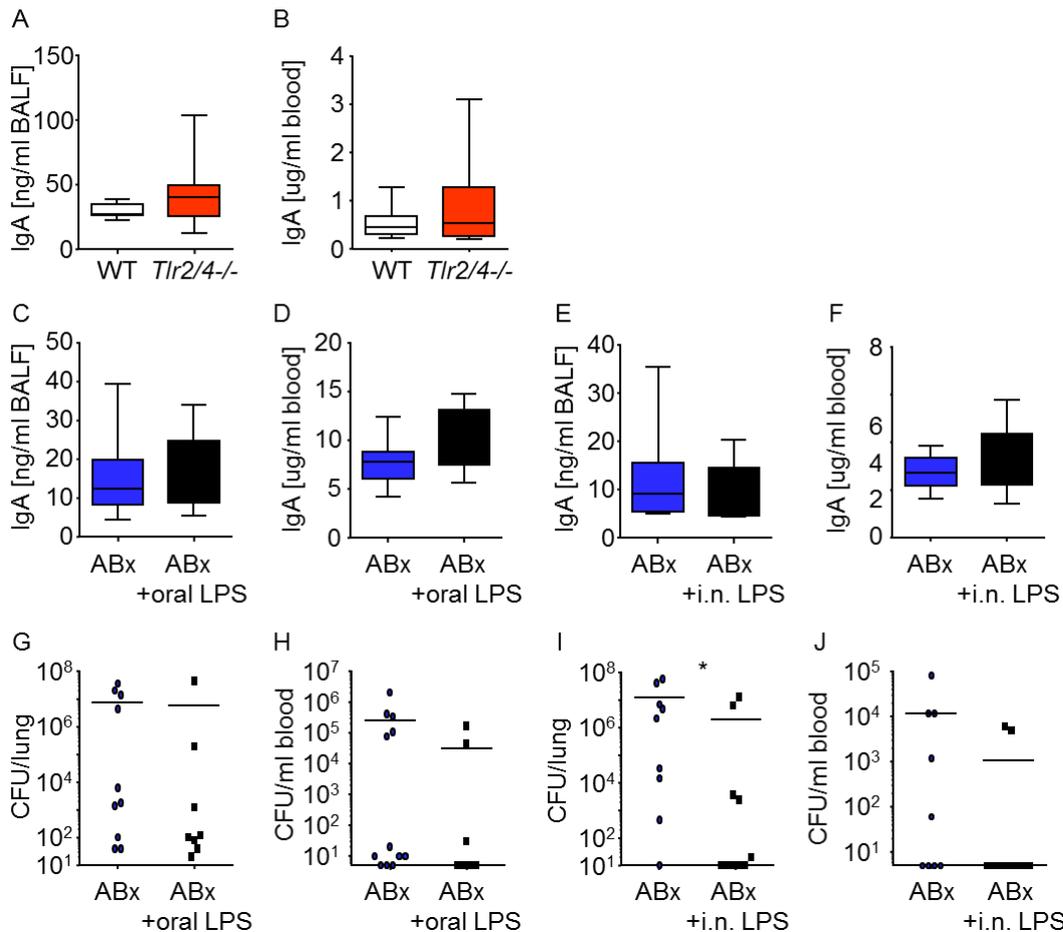


Supplemental Figure 1: Antibiotic treatment depletes the respiratory and intestinal microbiota.

(A) Total eubacterial bacterial loads of the lung microbiota (BALF) and upper respiratory tract microbiota (URL) of untreated (conv) and antibiotic-treated mice (ABx) were quantified by qPCR (relative quantification of 16S rRNA gene over PBS controls) (n = 5 for conv. mice; n = 2 for ABx mice). (B) The total eubacterial loads and (C) the main intestinal bacterial groups including enterobacteria, enterococci, lactobacilli, bifidobacteria, *Bacteroides/Prevotella* species, *Clostridium leptum* group, *Clostridium coccoides* group and *Mouse Intestinal Bacteroides* were assessed in fecal samples derived from conventionally colonized (Conv) and antibiotic-treated (ABx) mice applying qPCR amplifying variable regions of the bacterial 16S rRNA genes (expressed as 16S rRNA gene numbers per ng DNA) (n = 6 for conv. mice; n = 5 for ABx mice).



Supplemental Figure 2: Antibacterial immunity against *P. aeruginosa* is strongly impaired in germ-free as compared to conventionally colonized mice. Conventionally colonized and germ-free mice were intranasally infected with *P. aeruginosa* 10^5 CFU/mouse, and (A) bacterial loads in the lung and (B) blood were quantified after 24 h (n = 12 for conv. mice; n = 8 for germ-free mice).



Supplemental Figure 3: TLR2 and-4 are not involved in IgA production and LPS application does not restore IgA production in antibiotic-treated mice. (**A, B**) IgA levels were measured in BALF and blood of WT and *Tlr2/4*^{-/-} mice. (**C-E**) IgA levels were determined in BALF and blood of microbiota-depleted animals (ABx), microbiota-depleted animals that received LPS orally (ABx + oral LPS) or intranasally (ABx + i.n. LPS). (**C, D**, n = 12 for ABx, n = 11 for ABx + oral LPS; **E, F**, n = 9 for ABx, n = 10 for ABx + i.n. LPS). (**G-J**) Microbiota-depleted animals (ABx), microbiota-depleted animals that received LPS orally (ABx + oral LPS), and microbiota-depleted mice that received LPS intranasally (ABx + i.n. LPS) were infected with *P. aeruginosa*. Bacterial loads in lung and blood were assessed (**G**, n = 10 for ABx, n = 10 for ABx + oral LPS; **H**, n = 12 for ABx, n = 9 for ABx + oral LPS; **I**, n = 9 for ABx, n = 10 for ABx + i.n. LPS; **J**, n = 9 for ABx, n = 10 for ABx + i.n. LPS). Mann-Whitney tests were applied to the data set. Values are given as mean \pm standard deviation. *p < 0.05.