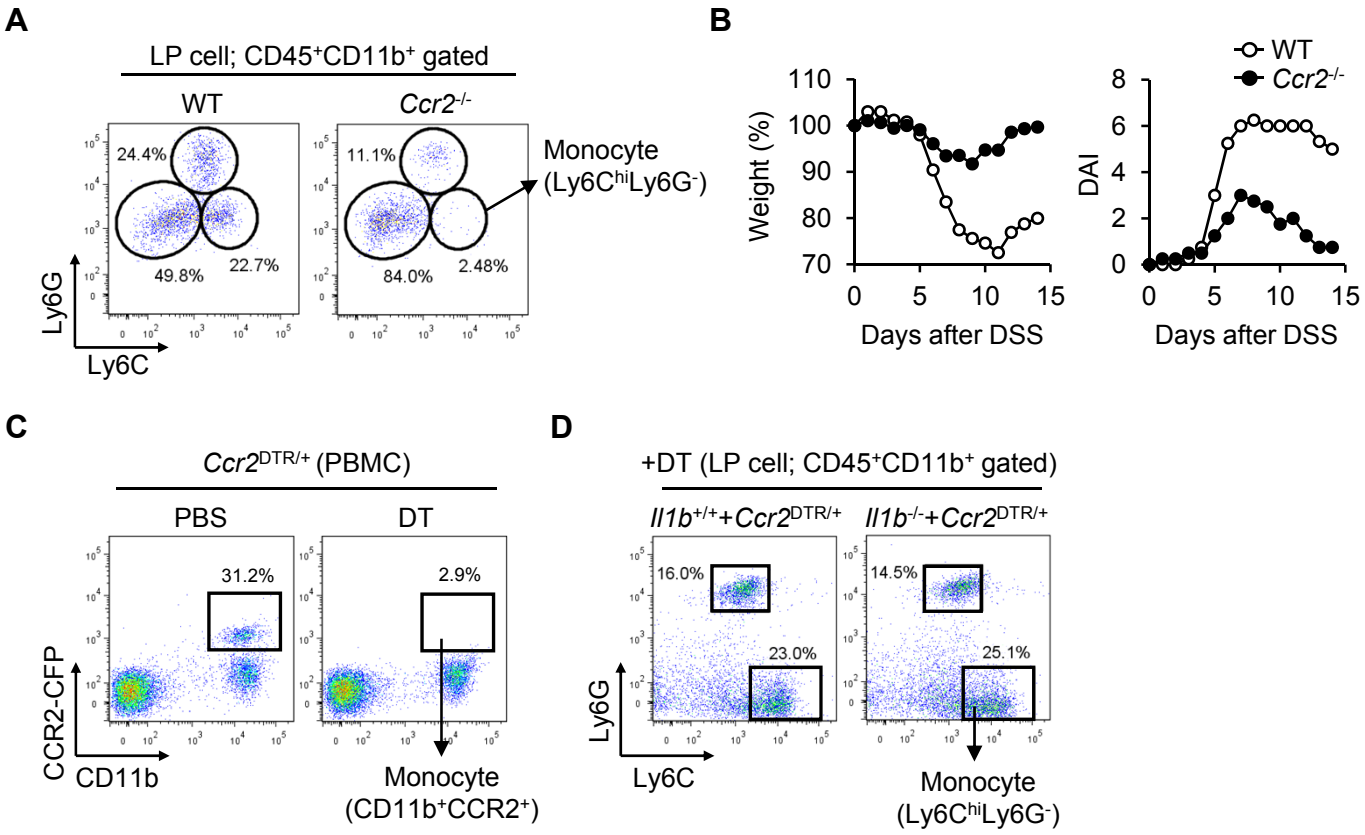


Figure S1, related to Figure 4. Analysis of *Ccr2*$^{-/-}$ mice and mixed chimeric mice with deletion of IL-1β in CCR2^{+} cells.

(A) *Ccr2*$^{-/-}$ mice fail to recruit Ly6C^{high} monocytes to the intestine on 7 days after DSS treatment.

(B) *Ccr2*$^{-/-}$ mice exhibit less weight loss and lower DAI scores than wild-type (WT) mice after 2% DSS treatment for 6 days.

(C) *Ccr2*$^{DTR/+}$ mice were treated with PBS or DT and depletion of monocytes was confirmed 2 days after treatment.

(D) Both *Il1b*$^{+/+}$+*Ccr2*$^{DTR/+}$ and *Il1b*$^{-/-}$+*Ccr2*$^{DTR/+}$ mixed chimeric mice were treated with 2.5% DSS and received DT on days 0, 3, and 6. The percentage of monocytes (CD11b^{+}Ly6C^{high}Ly6G^{-}) was comparable between *Il1b*$^{+/+}$+*Ccr2*$^{DTR/+}$ and *Il1b*$^{-/-}$+*Ccr2*$^{DTR/+}$ mice on day 7.

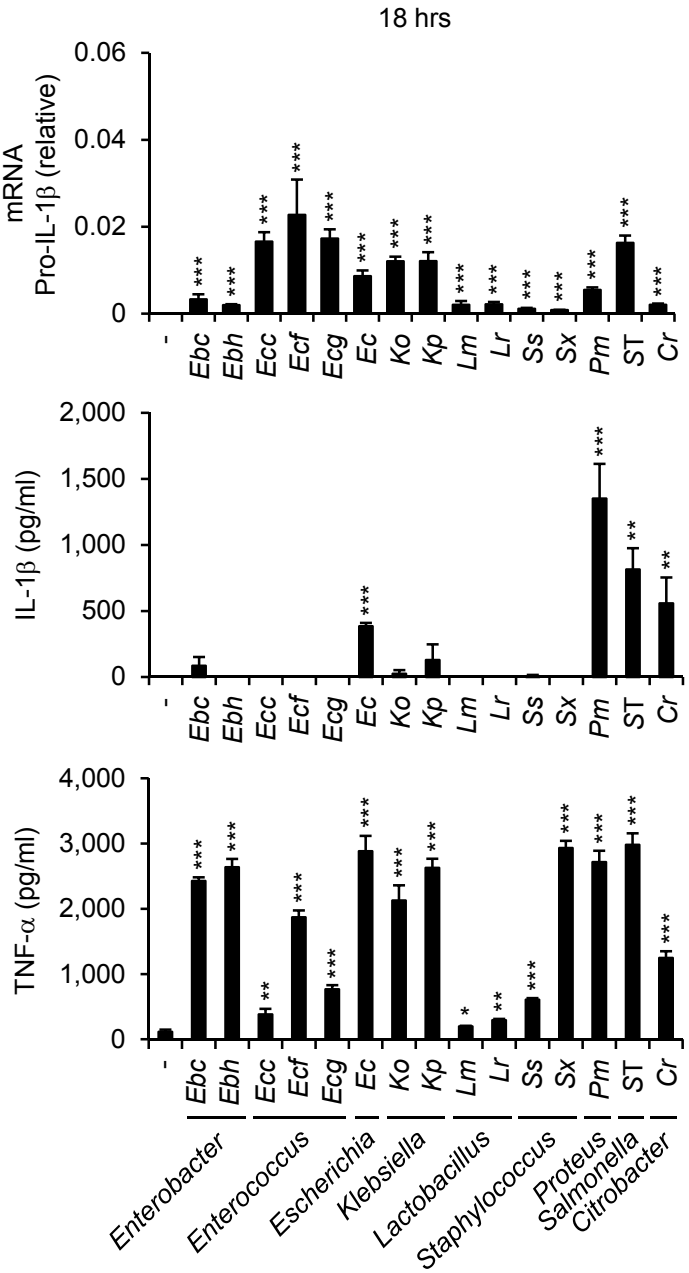


Figure S2, related to Figure 5. Selective commensals induce IL-1β release.

BMDM were stimulated with indicated mouse commensal bacteria for 18 hrs. Cytokine amounts in culture supernatant and pro-IL-1β mRNA levels were determined by ELISA and qRT-PCR, respectively. Data are representative of two experiments. Values present means of triplicated samples ± SD. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

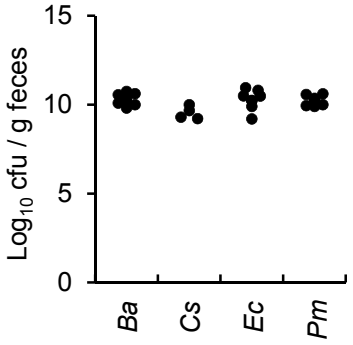


Figure S3, related to Figure 5. Bacteria counts in feces from germ-free mice mono-associated with commensal bacteria.

Germ-free mice were mono-associated with indicated mouse commensal bacteria. *Ba*, *Bacteroides acidifaciens*; *Cs*, *Clostridium sporogenes*; *Ec*, *Escherichia coli*, or *Pm*, *Proteus mirabilis*. Fecal samples were obtained 3 days after colonization and spread at different dilutions on blood agar plates (*Ba, Cs*) or MacConkey agar plates (*Ec, Pm*). Values represent samples from individual mice and results were pooled from three experiments with similar results.

A

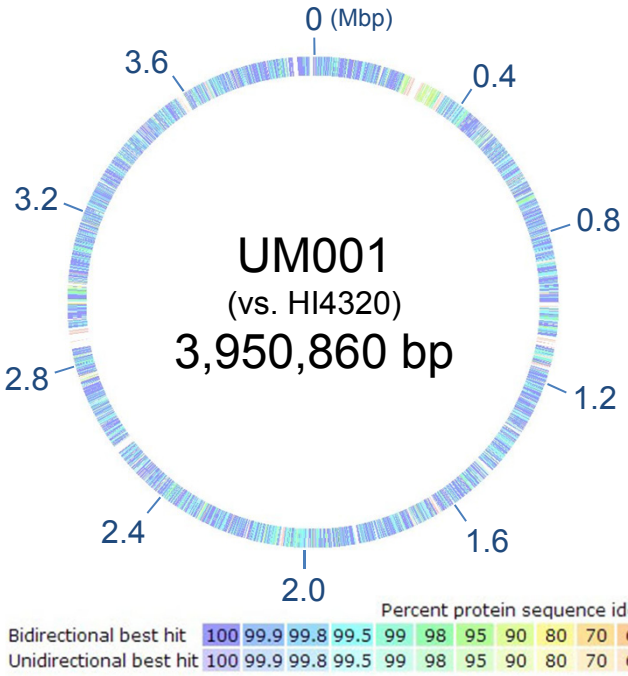


Figure S4, related to Figure 6. Genome and virulence gene comparison between mouse and human *P. mirabilis*.

(A) Schematic view of gene sequences in genomes from mouse *P. mirabilis* (UM001) and human reference strain HI4320.

(B) Amino acid sequence comparison of virulence factors between UM001 and HI4320.

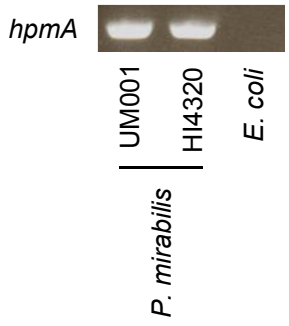
(C) The *hpmA* hemolysin gene were amplified by PCR from genomic DNA isolated from UM001 and HI4320 using the primers hpmA(F); 5'-GTTGAGGGGGCGTTATCAAGAGTC, hpmA(R); 5'-GATAACTGTTTTGCCCTTTTGTGC.

(D) Comparison of amino acid sequence of HpmA between UM001 (mouse) and HI4320 (human).

B

Gene	Function	Homology
cheW	Chemotaxis	166/166 (100%)
fliF	Flagellin	568/573 (99%)
hpmA	Hemolysin	1567/1577 (99%)
mrpA	Fimbria	175/175 (100%)
spa47	T3SS	404/409 (99%)
ureC	Urease	566/567 (99%)

C



D

HpmA; Identities 1567/1577 (99%), Positives 1573/1577 (99%)

```
Mouse   1    MKSKNFKLSPSGRLAASLAIIFVSLNAYGNGIVPDAGHQGPDVSAVNGGTQVINIVTPNN 60
Human   1    MKSKNFKLSPSGRLAASLAIIFVSLNAYGNGIVPDAGHQGPDVSAVNGGTQVINIVTPNN 60

Mouse   61   EGISHNQYQDFNVGKPGAVFNNALEGGQSQLAGHLNANSNLNGQAASLILNEVVSRNPSF 120
Human   61   EGISHNQYQDFNVGKPGAVFNNALEAGQSQLAGHLNANSNLNGQAASLILNEVVSRNPSF 120

Mouse   121  LLGQQEVFGIAAEYVLSNPNGITCDGCGFINTSRSSLVVGNPLFENGQLKGYSTLNNTNL 180
Human   121  LLGQQEVFGIAAEYVLSNPNGITCDGCGFINTSRSSLVVGNPLFENGQLKGYSTLNNTNL 180

Mouse   181  LSLGKNGLNTTGLLDLIAPRIDSRGKITAAEISAFTGQNTFSQHFDILSSQKPVSALDSY 240
Human   181  LSLGKNGLNTTGLLDLIAPRIDSRGKITAAEISAFTGQNTFSQHFDILSSQKPVSALDSY 240

Mouse   241  FFGSMQSGRIRIINTAEGSGVKLAGKFTADNDLSVKADNIQTDSQVRYDSYDKDGSENYQ 300
Human   241  FFGSMQSGRIRIINTAEGSGVKLAGKFTADNDLSVKADNIQTDSQVRYDSYDKDGSENYQ 300

Mouse   301  NYRGGITVNNSGSSQTLTKTELKGKNITLVASNHNQIKASDLMGDDITLQGADLTIDGKQ 360
Human   301  NYRGGITVNNSGSSQTLTKTELKGKNITLVASSHNQIKASDLMGDDITLQGADLTIDGKQ 360

Mouse   361  LQQKETDIDNRWFYSWKYDVTKEKEQIQQIGSQIDAKNNATLTATKGDVTLDAAKINAGN 420
Human   361  LQQKETDIDNRWFYSWKYDVTKEKEQIQQIGSQIDAKNNATLTATKGDVTLDAAKINAGN 420

Mouse   421  NLAINANKDIHINGLVEKESRSENGNKRNHTSRLESGSWSNSHQTETLKASELTAGKDLG 480
Human   421  NLAINANKDIHINGLIEKESRSENGNKRNHTSHLESGSWSNSHQTETLKASELTAGKDLG 480

Mouse   481  LDAQGSITAQGAKLHANENVLVNAKDNINLNVQKTNNDKTVTDNHVMWGGIGGGQNKNNN 540
Human   481  LDAQGSITAQGAKLHANENVLVNAKDNINLNVQKTNNDKTVTDNHVMWGGIGGGQNKNNN 540

Mouse   541  NQQQVSHATQLTADGQLLLAADNNVNITGSQVKGNQGAFVKTTQGDVVIDNAMSETISKI 600
Human   541  NQQQVSHATQLTADGQLLLAADNNVNITGSQVKGNQGAFVKTTQGDVVIDNALSETISKI 600

Mouse   601  DERTGTAFNITKSSHKNETNKQTSTGSELISDAQLTVVSGNDVNVIGSLIKSADKLGIHS 660
Human   601  DERTGTAFNITKSSHKNETNKQTSTGSELISDAQLTVVSGNDVNIIGSLIKSADKLGIHS 660

Mouse   661  LGDINVKSAQQVTKIDDEKTSLAITGHAKEVEDKQYSAGFHITHTTNKNTSTETEQANST 720
Human   661  LGDINVKSAQQVTKIDDEKTSLAITGHAKEVEDKQYSAGFHITHTTNKNTSTETEQANST 720

Mouse   721  ISGANVDLQANKDVTFAGSDLKTTAGNASITGDNVAFVSTENKKQADNTDTTISGGFSYT 780
Human   721  ISGANVDLQANKDVTFAGSDLKTTAGNASITGDNVAFVSTENKKQADNTDTTISGGFSYT 780

Mouse   781  GGVDKVGSKADFQYDKQHTQTEVTKNRGSQTEVAGDLTITANKDLLHEGASHHVEGRYQE 840
Human   781  GGVDKVGSKADFQYDKQHTQTEVTKNRGSQTEVAGDLTITANKDLLHEGASHHVEGRYQE 840

Mouse   841  SGENIQHLAVNDSETSKTDSLNVGIDVGVNLDYSGVTKPVKKAIEDGVNTTKPGNNTDLT 900
Human   841  SGENIQHLAVNDSETSKTDSLNVGIDVGVNLDYSGVTKPVKKAIEDGVNTTKPGNNTDLT 900

Mouse   901  KKVTARDAIANLANLSNLETPNVGVEVGIKGGGSQKSKTDSQAVSTSINAGKINIDSNNK 960
Human   901  KKVTARDAIANLANLSNLETPNVGVEVGIKGGGSQKSQTDSQAVSTSINAGKINIDSNNK 960

Mouse   961  LHDQGTHYQSTQEGISLTANTHTSEAAQDKHQTTFHETKGGGQVGVSTKTGSDITVAIKG 1020
Human   961  LHDQGTHYQSTQEGISLTANTHTSEVAQDKHQTTFHETKGGGQVGVSTKTGSDITVAIKG 1020

Mouse   1021 EGQTTDNALMETKAKGSQFTSNGDISINVGEDAHYEGAQFDAQKGKTVINAGGDLTLAQA 1080
Human   1021 EGQTTDNALMETKAKGSQFTSNGDISINVGEDAHYEGAQFDAQKGKTVINAGGDLTLAQA 1080

Mouse   1081 TDTHSESQSNVNGSANLKVGTTPESKDYGGGFNAGTTHHSKEQTTAKVGAITGSQGIELN 1140
Human   1081 TDTHSESQSNVNGSANLKVGTTPESKDYGGGFNAGTTHHSKEQTTAKVGAITGSQGIELN 1140

Mouse   1141 AGHNLTLQGTHLSSEQDIALNATNKVDLQSASSERTEKGNNLSGGVQAGFGKKMTDDASS 1200
Human   1141 AGHNLTLQGTHLSSEQDIALNATNKVDLQSASSERTEKGNNLSGGVQAGFGKKMTDDASS 1200

Mouse   1201 VNGLGSAQFAIGKQDEKSVSREGGTINNSGNLTINGNSVHLQGAQVNSKDTQLTSQSGDI 1260
Human   1201 VNGLGSAQFAIGKQDEKSVSREGGTINNSGNLTINGNSVHLQGAQVNSKDTQLTSQSGDI 1260

Mouse   1261 EITSAQSTDYKNNWGTDIGFNGKKTNTTPKEVTEEKPATSIHNIGGKLLVNVEDQQKTSH 1320
Human   1261 EITSAQSTDYKNNWGTDIGFNGKKTNTTPKEVTEEKPATSIHNIGGKLLVNVEDQQKTSH 1320

Mouse   1321 QNATLETGTLTINSNKDLTLSGANVTADSVTGNVGGSLNIASQKESDRHVTVGVNVGYNH 1380
Human   1321 QNATLETGTLTINSNKDLTLSGANVTADSVTGNVGGSLNIASQKESDRHVTVGVNVGYNH 1380

Mouse   1381 TNDPKSSQVNKTAKAGGSLLEKTIKDTIDSGIKSSTDAISDKYNSLSSTIADKTGISDET 1440
Human   1381 TNDPKSSQVNKTAKAGGSLLEKTIKDTIDSGIKSSTDAISDKYNSLSSTIADKTGISDET 1440

Mouse   1441 KAKIDQGFGKVGNGIKNIVTGAEGHTANADIKVTHVDNDAVTKTTSLTSNNDLSLNVNGS 1500
Human   1441 KAKIDQGFGKVGNGIKNIVTGAEGHTANADIKVTHVDNDAVTKTTSLTSNNDLSLNVNGS 1500

Mouse   1501 TKLTGAEIASQQGQVDLGGSSVKLENIEGHHYEAGADLDLKSSVVDLAKQLVGGDISFKS 1560
Human   1501 TKLTGAEIASKQGQVDLGGSSVKLENIEGHHYEAGADLGLKSSVVDLAKQLVGGDISFKS 1560

Mouse   1561 PVKTNETVNTKASISEK 1577
Human   1561 PVKTNETVNTKASISEK 1577
```

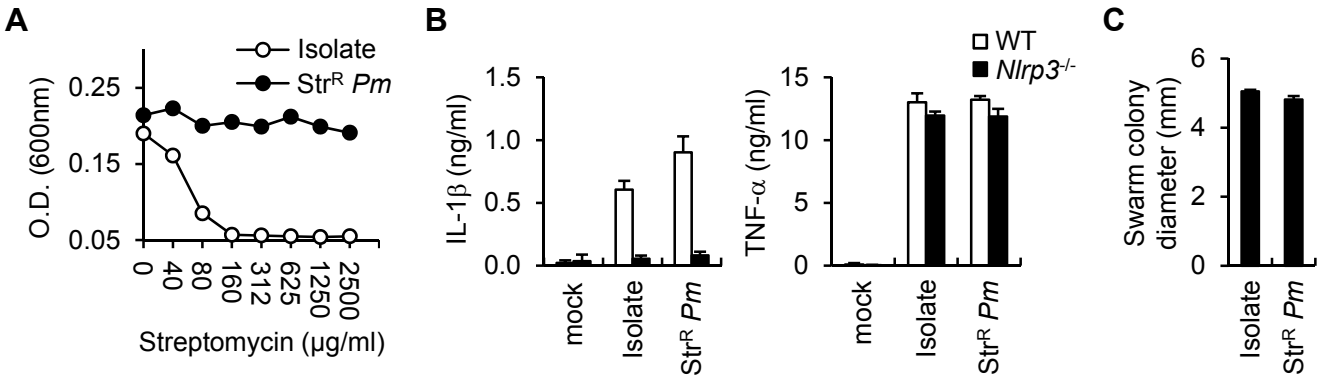


Figure S5, related to Figure 7. Generation of streptomycin-resistant *P. mirabilis* (Str^R *Pm*).

(A) Mouse-isolated *P. mirabilis* strain UM001 resistant to streptomycin (Str^R *Pm*) and parental strain (Isolate) were grown in LB medium containing different concentration of streptomycin.

(B) Parental strain (Isolate) and Str^R *Pm* strain were used to stimulate WT and *Nlrp3*$^{-/-}$ BMDM for 3 hrs. Cytokine levels were determined by ELISA.

(C) Parental strain (Isolate) and Str^R *Pm* strain were inoculated in LB broth for 6 hrs and 10 μl of inoculum was placed in the center of LB plate. Swarm colony diameter was measured after 18 hours of culture at 37°C.

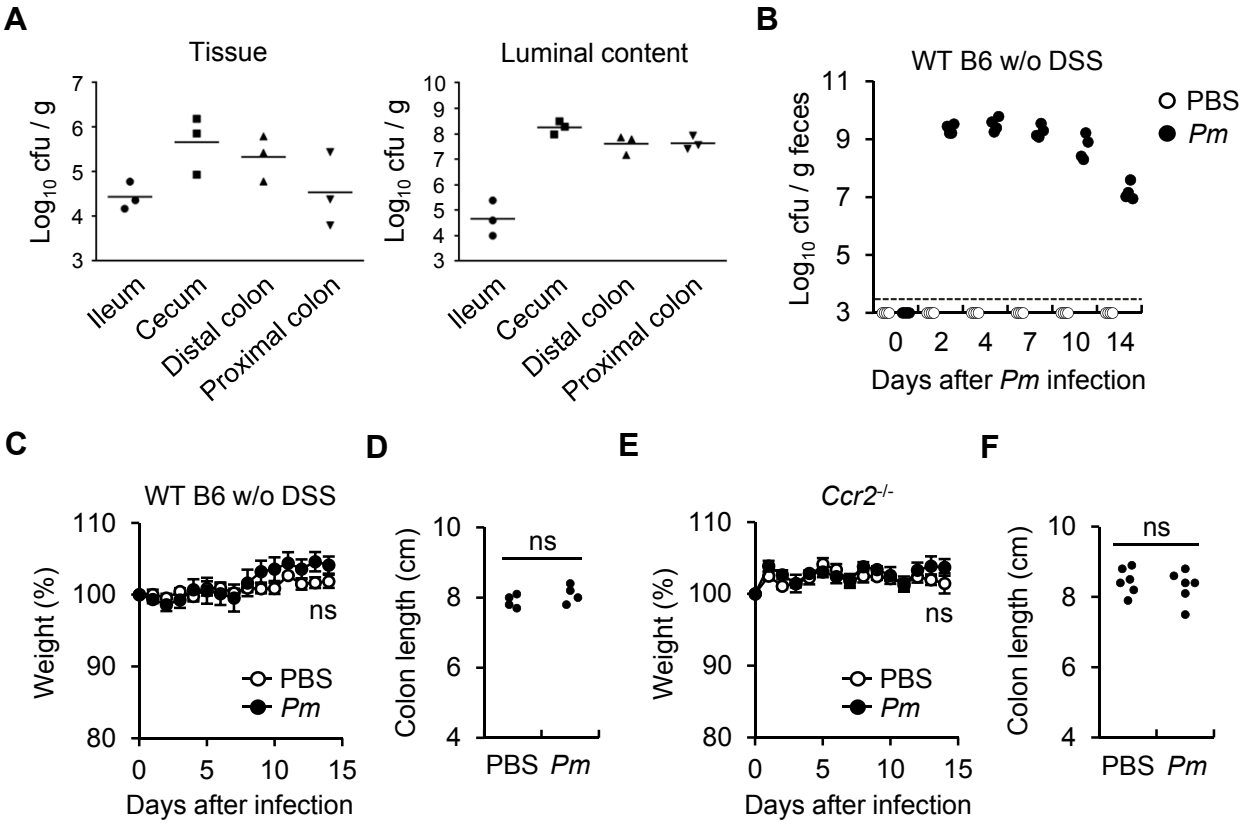


Figure S6, related to Figure 7. Colonization with *P. mirabilis* requires DSS treatment to exacerbate colitis.

(A) Mice were treated orally with 20 mg streptomycin and 24 hrs later the mice were gavaged with 1×10^9 cfu of streptomycin-resistant *P. mirabilis* (*Pm*). Bacteria numbers were counted from tissue and luminal contents on day 4 after infection (n = 3). Data are representative for two experiments.

(B)-(F) Mice were treated with streptomycin as in Figure 7A and gavaged with 10^9 cfu of streptomycin-resistant *P. mirabilis* (*Pm*). (B) Bacteria numbers in the feces of gavaged wild-type (WT) mice are shown. Bacterial burden in feces at indicated time points are shown (n = 4, each group). (C) Body weight of infected wild-type mice was monitored for 2 weeks. (D) Mice were sacrificed and colon length measured on day 15. (E) *Ccr2*$^{-/-}$ mice were gavaged with *Pm* on day 0, 4, 8. Mice were given 1% DSS from day 0 in the drinking water. Body weight was monitored for 2 weeks and intestinal inflammation was assessed on day 15. ns; not significant.

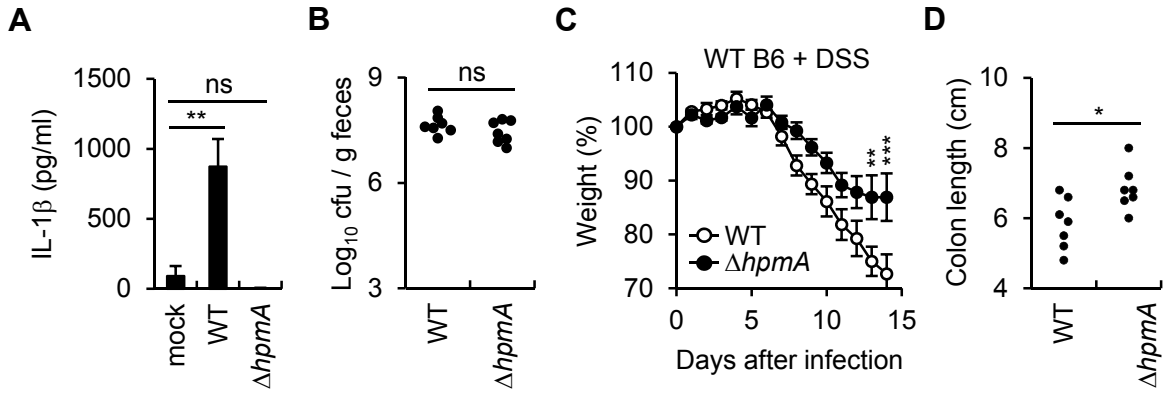


Figure S7, related to Figure 7. Hemolysin A of mouse-isolated *P. mirabilis* induces IL-1β production in BMDM and enhances DSS colitis.

(A) BMDM were stimulated with WT or hemolysin A-deficient mutant (Δ*hpmA*) streptomycin-resistant *P. mirabilis* for 3 hrs and IL-1β in culture supernatant were measured by ELISA. Means ± SD are shown. ***p* < 0.01, ns; not significant.

(B)-(D) Mice were treated with streptomycin as in Figure 7A and gavaged with 10^9 cfu of WT or Δ*hpmA* mouse-isolated streptomycin-resistant *P. mirabilis*. Mice were treated with 1% DSS from day 0 to 10 and infected with *P. mirabilis* at day 0, 4, 8 (n = 7, each group, pooled from 2 separate experiment with similar results). (B) Bacteria numbers in the feces of inoculated mice were determined on day 4. (C) Mouse body weight was monitored for 2 weeks. Means ± SEM are shown. (D) Mice were sacrificed on day 15 and colon lengths were measured. **p* < 0.05, ***p* < 0.01.