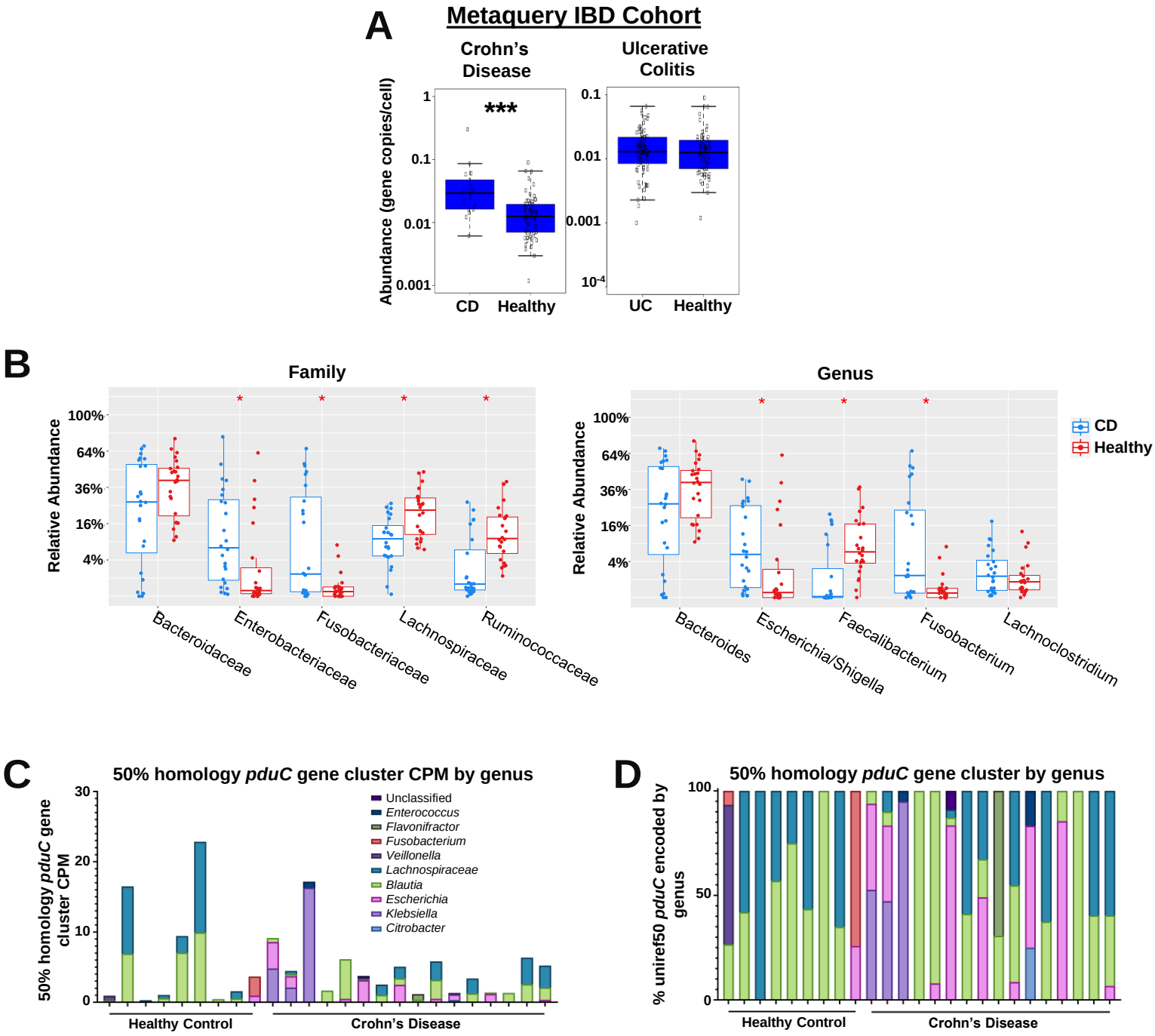



**Fig S1. *pduC* is enriched in CD microbiome, *related to Figure 1.*** **A.** Gene abundance of *pduC* from patients with Crohn's disease (CD), ulcerative colitis (UC), or healthy controls from metaquery database (McGovern et al., 2010). **B.** Composition of fecal microbiome from 16S rRNA sequencing of healthy controls (HC, N=24) and Crohn's disease (CD, N=24). Top five abundant families and genus are shown. **p*<0.05 by Mann-Whitney. **C.** Read counts per million of *pduC* based on 50% homology. Reads encoded by genus are indicated by color. **D**. Percentage of *pduC* reads encoded by genus.

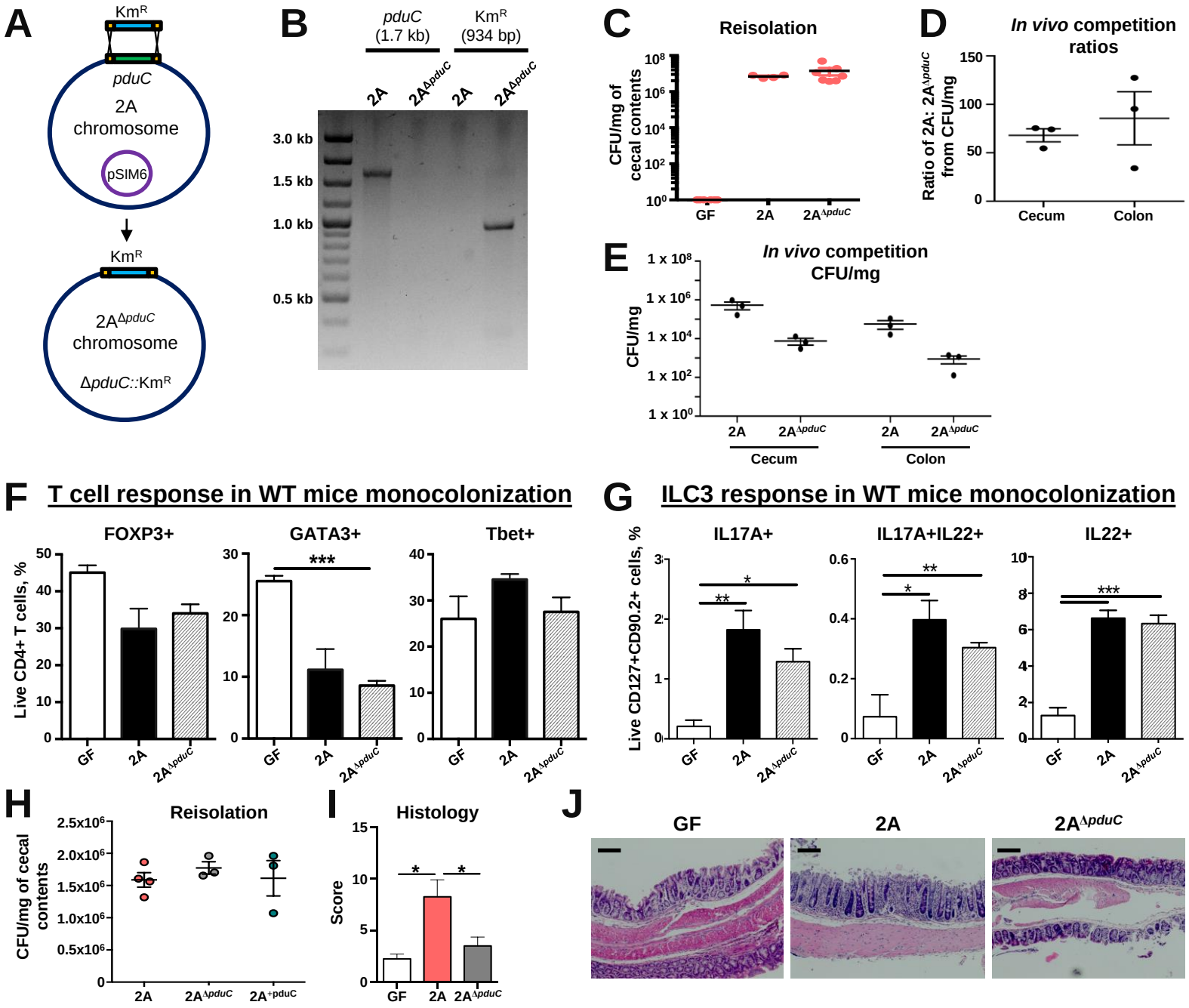



**Fig S2. CD-derived AIEC induces intestinal RORγt immunity and inflammation in a *pduC*-dependent manner, *related to Figure 1.*** **A.** Lamba red recombination was used to generate a CD-derived, *pduC*-deficient AIEC mutant (**2A**$^{\Delta pduC}$) **B.** 1.7% agarose DNA gel for PCR amplified *pduC* and kanamycin resistance cassette (Km$^R$) in 2A and 2A$^{\Delta pduC}$. **C.** Germ-free C57BL/6 mice were colonized with 2x10$^9$ CFU AIEC 2A or 2A$^{\Delta pduC}$. Analysis of CFU / mg colonic contents at day 15 post-colonization is shown. Each dot represents an individual mouse from one of two total experiments. Error bars represent SEM. **p*<0.05, ***p*<0.01 ANOVA. **D, E.** *In vivo* competitive ratios (**D**) and CFU (**E**) of wildtype AIEC 2A to 2A$^{\Delta pduC}$. Ratios were calculated based on CFU/mg counts from differential, selective plating on MacConkey agar with and without Kanamycin. **F, G.** Germ-free C57BL/6 mice were colonized with 2x10$^9$ CFU AIEC 2A or 2A$^{\Delta pduC}$ and analyzed after 15 days. Percentage of colonic FoxP3+, GATA3+, and Tbet+ CD4+ T cells was evaluated (**F**)**.** Percentage of colonic IL17A+, IL17A/IL22+ and IL22+ CD127+CD90.2+ ILC cells was evaluated (**G**)**.** Bar graphs represent geometric mean of at least 3 mice per group from one of three total experiments. Error bars represent SEM. **p*<0.05, ***p*<0.01 ANOVA. **H-J.** Germ-free *Rag1*-deficient mice were colonized with 2x10$^9$ CFU of AIEC 2A, 2A$^{\Delta pduC}$ or complement 2A$^{\Delta pduC+pduC}$. One week after colonization, mice received 500,000 FACS-sorted, naïve CD4+ T cells intraperitoneally. Analysis of CFU / mg colonic contents at week 10 post-T cell transfer is shown (**H**). Proximal colon was collected at week 10 for histopathological analysis (**I**). Representative colonic histology images are shown. Scale bars: 100μm (**J**).

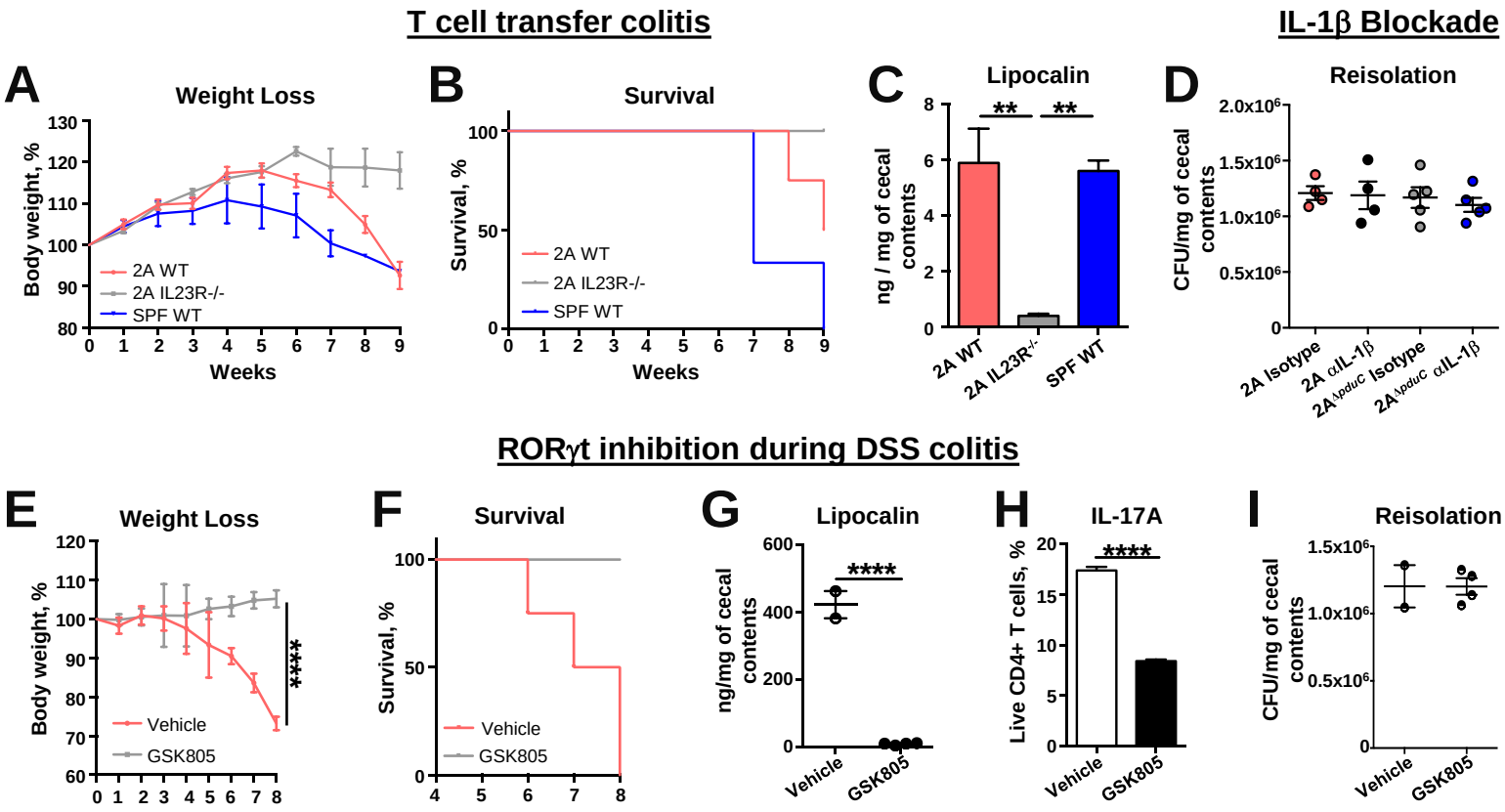



**Fig S3. CD-derived AIEC induces Th17-dependent intestinal inflammation, *related to Figures 1 and 2.*** **A-C.** Germ-free *Rag1*-deficient mice were colonized with 2x10$^9$ CFU of AIEC 2A. One week after colonization, mice received 500,000 FACS-sorted, naïve WT or *il23r*-deficient CD4+ T cells intraperitoneally. Mice were monitored for weight loss (**A**) and survival (**B**) for 9 weeks. Lipocalin in cecal contents was measured by ELISA at 9 weeks (**C**). Graphs represent geometric mean of 4-5 mice per group. Error bars represent SEM. ***p*<0.01 ANOVA. **D.** Germ-free C57BL/6 *Il10*-deficient mice were colonized with 2x10$^9$ CFU of AIEC 2A or 2A$^{\Delta pduC}$. Ten days after colonization, mice were exposed to 2% dextran sodium sulfate *ad libitum* for 7 days. Mice were treated intraperitoneally with anti-IL1β or isotype control on days -2, 0, 2, 4, and 6 of DSS treatment. Bacterial reisolation from cecal contents is shown. Each dot represents an individual mouse from one of two total experiments. Error bars represent SEM. **p*<0.05, t-test. **E-I.** Germ-free C57BL/6 *Il10*-deficient mice were colonized with 2x10$^9$ CFU of AIEC 2A. Ten days after colonization, mice were exposed to 2% dextran sodium sulfate *ad libitum* for 7 days. Mice were treated daily with RORγt inhibitor GSK805 or vehicle control. Weight loss (**E**), percent survival (**F**), levels of lipocalin in cecal contents (**G**) and colonic IL17A-producing CD4+ T cells (**H**), and bacterial reisolation (**I**) are shown. Graphs show at least 4 mice from one of two total experiments. Error bars represent SEM. *****p*<0.001 ANOVA.

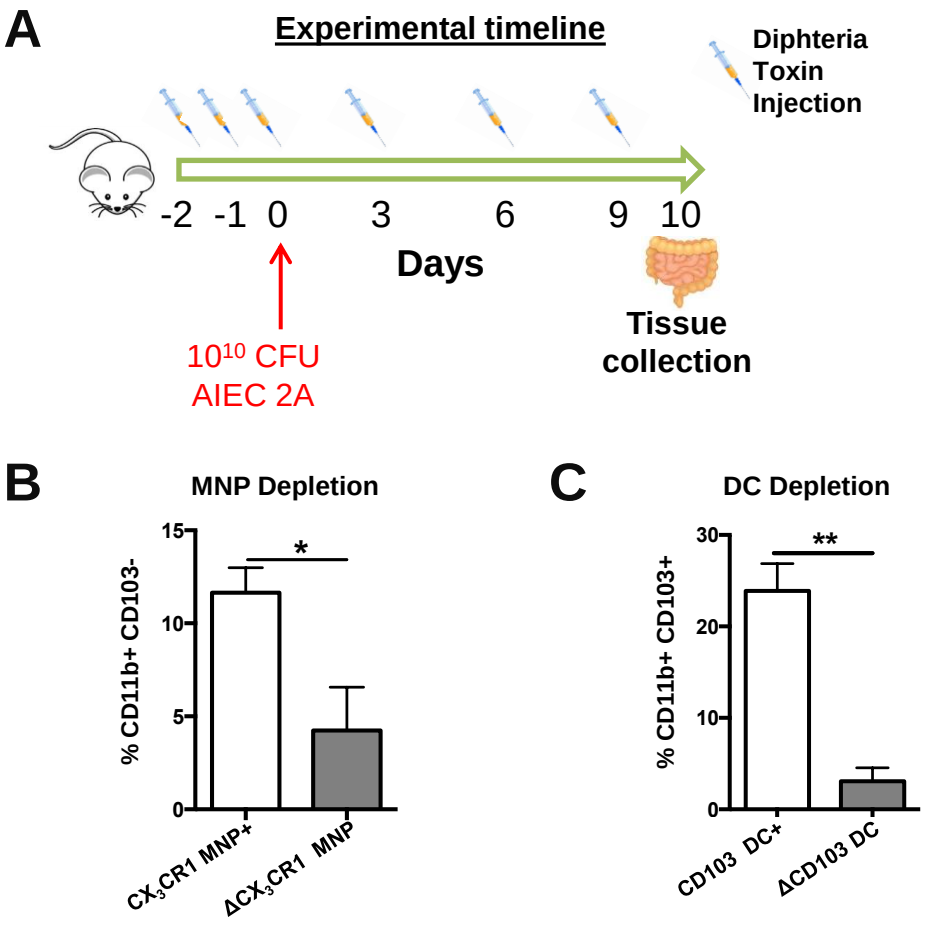
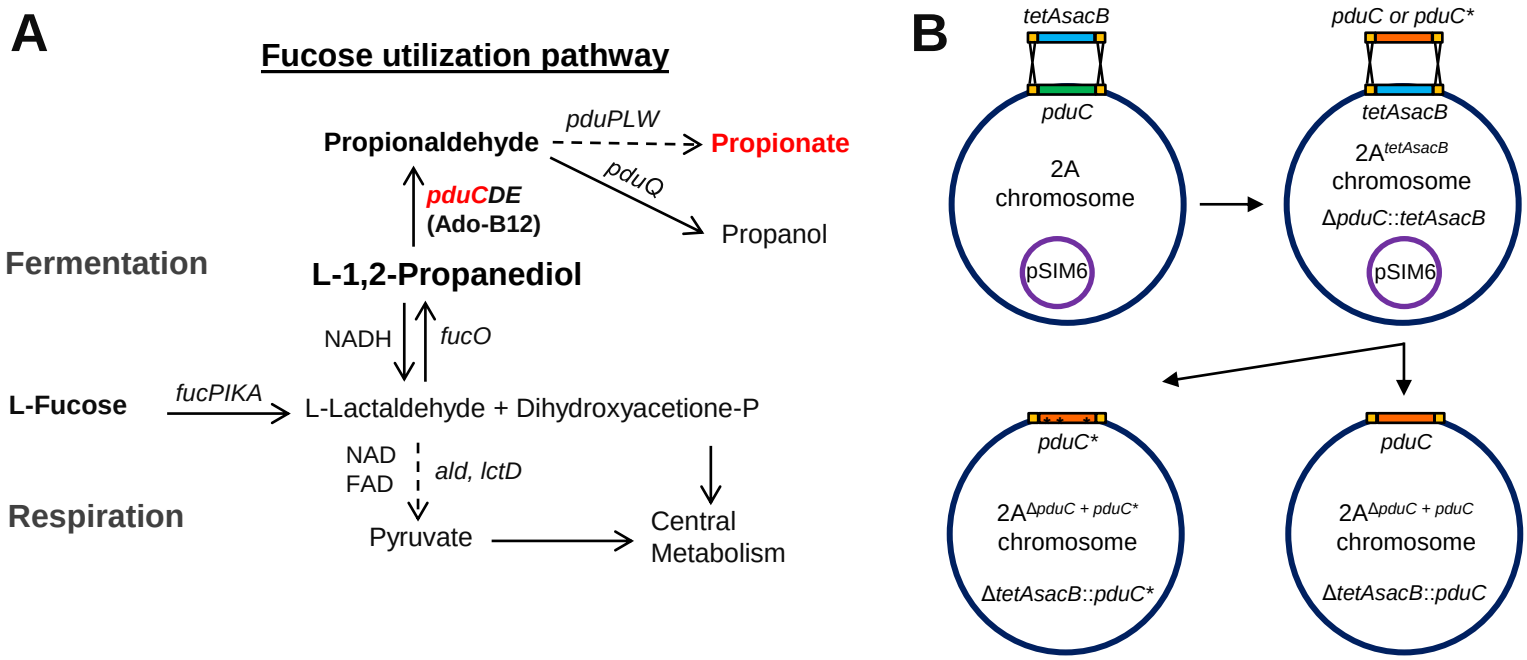
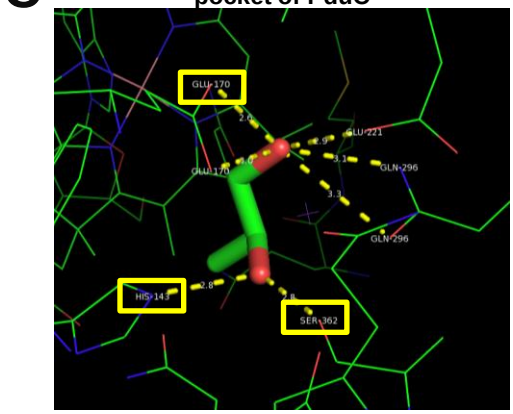
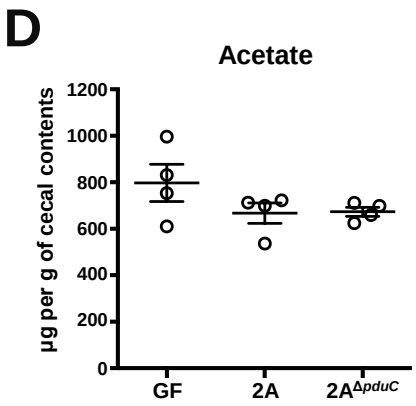
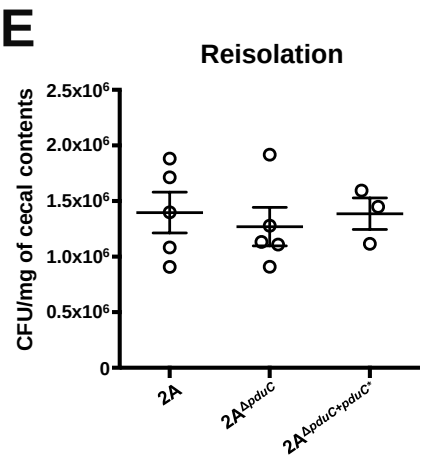


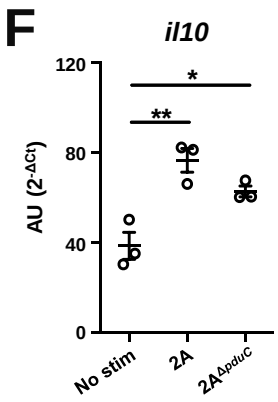

**FIGURE S4. Antigen presenting cell depletion strategy, *related to Figure 3.*** **A.** 4-week-old littermate *Cx3cr1-LSL-DTR* (labelled $CX_3CR1$ MNP+) and *Itgax-cre Cx3cr1-LSL-DTR* (labeled ΔCX3CR1 MNP) mice were treated with diphtheria toxin (DT) before and during colonization with $10^{10}$ CFU CD-derived AIEC 2A. 4-week-old littermate *Lang-DTREGFP* mice were treated with DT (labeled ΔCD103 DC) or PBS (labeled CD103 DC+) before and during colonization with AIEC 2A. **B, C.** Flow cytometry of ileal live, CD11c+ MHCII+ cells was used to evaluate depletion of CD11b+CD103- (MNPs) in **B** and CD11b+CD103+ (DCs) in **C**. Bar graphs represent geometric mean of at least 3-4 mice per group from one of three total experiments. Error bars represent SEM. **p*<0.05, ***p*<0.01, ANOVA.



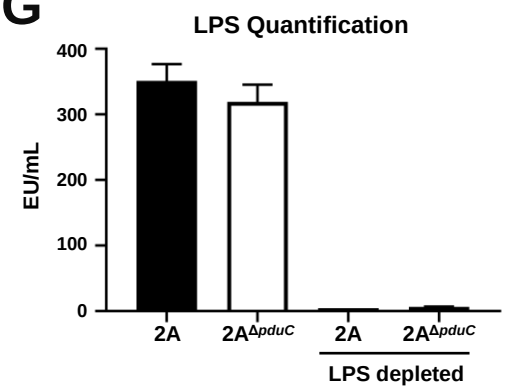




**Fig S5. PduC metabolic activity is required for T cell-dependent inflammatory colitis, *related to Figures 4 and 5.*** **A.** Fucose utilization pathway. **B.** 2A$^{pduC*}$ was generated using QuikChange Lightning Multi Site-directed Mutagenesis Kit (Agilent Technologies). **C.** 1,2-propanediol in the binding pocket of PduC. Targeted residues to create catalytically-inactive, isogenic mutant PduC* are highlighted by yellow boxes. **D.** Germ-free C57BL/6 WT mice were colonized with 2x10$^9$ CFU of AIEC 2A or 2A$^{\Delta pduC}$ for 5 days and cecal acetate levels were measured. Each dot represents a mouse from one experiment. Error bars represent SEM. *$p$<0.05, ANOVA. **E.** Germ-free *Rag1*-deficient mice were colonized with 2x10$^9$ CFU of AIEC 2A, 2A$^{\Delta pduC}$ or 2A$^{pduC*}$. One week after colonization, mice received 500,000 naïve CD4+ T cells I.P. Analysis of CFU/mg cecal contents is shown. Each dot represents an individual mouse from one of two total experiments. Error bars represent SEM. *$p$<0.05, ANOVA. **F,G.** AIEC 2A or 2A$^{\Delta pduC}$ were grown anaerobically in M9-minimal media containing 20 mM L-fucose as the sole carbon source for 72 hours. Cell-free supernatants were used to stimulate bone marrow-derived macrophages (BMDM) for 16h. *Il10* expression was measured by quantitative PCR. Graph shows 3 samples per group from one of three experiments. Error bars represent SEM. *$p$<0.05, **$p$<0.01 ANOVA (**F**). LPS was depleted from cell-free bacterial supernatants using the Pierce$^{Tm}$ endotoxin-binding resin in spin-column format. LPS was measured spectrophotometrically using the amebocyte lysate assay (**G**).

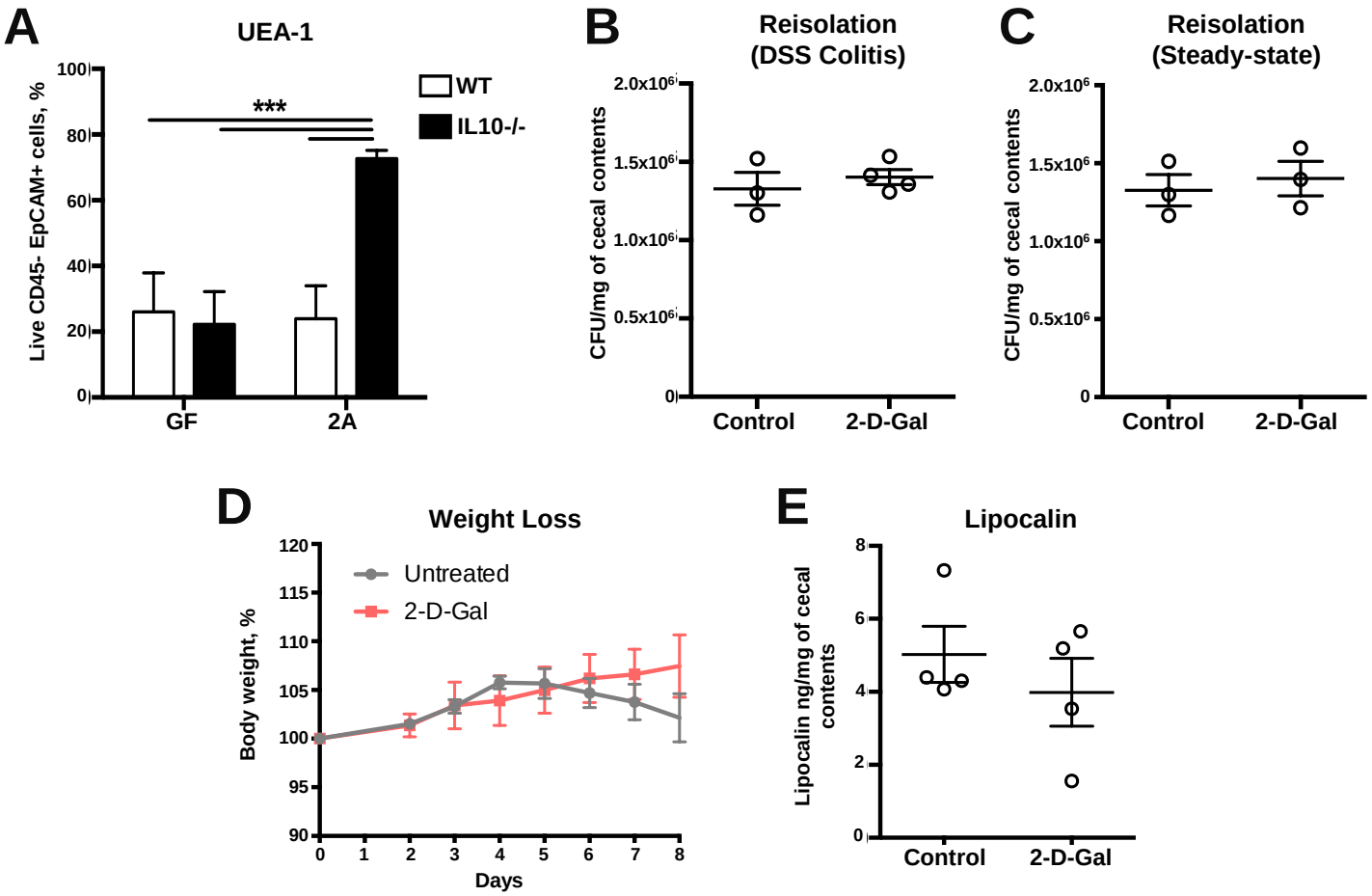



**Fig S6. Regulation of mucosal fucose mediates AIEC pathogenesis in colitis, *related to Figure 6.*** **A.** Germ-free C57BL/6 and *Il10*-deficient mice were colonized with 2x10$^9$ CFU AIEC 2A or 2A$^{\Delta pduC}$ for 4 days. Ileal epithelial cells were stained for CD45, EpCAM and UEA-I. CD45-EpCAM+ cells were gated and analyzed for UEA-I binding by flow cytometry. Bar graphs represent 4 mice per group. Error bars represent SEM. ***$p$<0.005 ANOVA. **B.** Germ-free C57BL/6 *Il10*-deficient mice were colonized with 2x10$^9$ CFU of AIEC 2A. Ten days after colonization, mice were exposed to 2% dextran sodium sulfate *ad libitum* for 7 days. Mice were treated intraperitoneally with 2-Deoxy-D-galactose or PBS control on days -2, 0, 2, 4, and 6 of DSS treatment. Bacterial reisolation from cecal contents is shown. **C.** C57BL/6 B6 SPF mice were treated with broad spectrum antibiotics for 2 weeks prior to infection with 10$^{10}$ CFU of AIEC 2A. Mice were treated intraperitoneally with 2-Deoxy-D-galactose or PBS control on days -2, 0, 2, and 4 after infection. Bacterial reisolation from cecal contents is shown. Each dot represents an individual mouse from one of two experiments. Error bars represent SEM. *$p$<0.05, t-test. **D, E.** Germ-free C57BL/6 *Il10*-deficient mice were colonized with 2x10$^9$ CFU of AIEC 2A$^{\Delta pduC}$. Ten days after colonization, mice were exposed to 2% dextran sodium sulfate *ad libitum* for 7 days. Mice were treated intraperitoneally with 2-Deoxy-D-galactose (2-D-gal) or PBS control on days -2, 0, 2, 4, and 6 of DSS treatment. Percent weight loss (**D**) is shown. Levels of lipocalin in cecal contents was measured by ELISA (**E**). Graphs represent geometric mean of 4 mice per group from one of two total experiments.

**Table S1: Bacterial Strains, Related to STAR methods**

| Bacteria (*E. coli*) | Genotype | Reference/Source |
|---|---|---|
| AIEC 2A | Parent strain | (Viladomiu et al., 2017) |
| AIEC 2A$^{\Delta pduC}$ | 2A Δ*pduC*::Km$^{R}$ | This work |
| AIEC 2A Δ*pduC::tetAsacB* | 2A Δ*pduC*::*tetAsacB* | This work |
| AIEC 2A Δ*pduC::tetAsacB* (pSIM6) | 2A Δ*pduC*::*tetAsacB,* pSIM6 | This work |
| AIEC 2A$^{pduC*}$ | 2A Δ*pduC*::*tetAsacB*::*pduC*:427C>G, 428A>C, 509A>C, 1084T>G | This work |
| AIEC 2A$^{+pduC}$ | 2A Δ*pduC*::*tetAsacB*::*pduC* | This work |
| BL21(DE3) | F$^{-}$*ompT hsdS$_B$* ($r_B^-$ $m_B^-$) *gal dcm*(DE3); PduCDE expression | Novagen |
| T-SACK | W3110 *araD<>tetA-sacB-amp fliC<>cat argG*::*Tn*5; cassette amplification | Court lab, NCI |
| *Bacteroides thetaoitamicron* | Wild-type | (Benjdia et al., 2011) |

Strains of bacterial used or generated for this manuscript. Italics designate a gene.

**Table S2: Plasmids, Related to STAR methods**

| Plasmid | Genetic Info | Reference/Source |
|---|---|---|
| pSIM6 | *exo*, *bet*, *gam*, $Amp^R$, $repA^{Ts}$ | Court lab, NCI |
| pUC19 | PCR cloning vector | NEB |
| pET30b | PduCDE expression vector, $Km^R$ | Novagen |

Plasmids used for this manuscript. Italics designate a gene.

**Table S3: Oligonucleotides, Related to STAR methods**

| Primers | Sequence (5' → 3') | Purpose | Reference/ Source |
|---|---|---|---|
| Kan + pduC F | CGATACACCGAAAAACGATCGCCCTTCCTACATCTGAATTCTCTGAGGCAGATTTTATGGACAGCAAGCGAACCG | λRed Recombination, pdu homology for $Km^R$ amplification | This work |
| Kan + pduC R | GCACGCCTTCGATAATCTGACGCAGTAATTTTTCGTTAATTTCCATTTCTCACCCTCAGAAGAACTCGTCAAGAAGG | λRed Recombination, pdu homology for $Km^R$ amplification | This work |
| Kan + pduC ext F | CATCTCCGCACGCGAAATCGGTAAAACAGTGCTTTCGACTCTCGGCGATACACCGAAAAACGATC | pdu homology extension | This work |
| Kan + pduC ext R | GCACGAAATGAAACGGACTTGTCGCTGGTTTGCATTTCTGCCAGCACGCCTTCGATAATCTGAC | pdu homology extension | This work |
| TetA + pduC F | CACCGAAAAACGATCGCCCTTCCTACATCTGAATTCTCTGAGGCAGATTTTCCTAATTTTTGTTGACACTCTATC | λRed Recombination, pdu homology for *tetA-sacB* amplification | This work |
| TetA + pduC R | CCTTCGATAATCTGACGCAGTAATTTTTCGTTAATTTCCATTTCTCACCCATCAAAGGGAAAACTGTCCATATGC | λRed Recombination, pdu homology for *tetA-sacB* amplification | This work |
| TetA + pduC ext F | CGCGAAATCGGTAAAACAGTGCTTTCGACTCTCGGCGATACACCGAAAAACGATC | pdu homology extension | This work |
| TetA + pduC ext R | CGGACTTGTCGCTGGTTTGCATTTCTGCCAGCACGCCTTCGATAATCTGACG | pdu homology extension | This work |
| KmR F | TATGGACAGCAAGCGAACCG | $Km^R$ cassette detection | Court lab, NCI |
| KmR R | GAAGGCGATAGAAGGCG | $Km^R$ cassette detection | This work |
| TetA-SacB F | TCCTAATTTTTGTTGACACTC | *tetA-sacB* amplification/detection | Court lab, NCI |
| TetA-SacB R | ATCAAAGGGAAAACTGTCC | *tetA-sacB* amplification/detection | Court lab, NCI |
| pduC F | ATGAGATCGAAAAGATTTGAAGC | *pduC* amplification/detection | This work |
| pduC R | TTAGTCAATTTCGTTGGGATC | *pduC* amplification/detection | This work |
| RS-pduC F | GAAATCAAGCTTGGTAAAACAGTGCTTTCGAC | Amplify *pduC* with HindII restriction site for incorporation into pUC19 | This work |
| RS-pduC R | CGGAGGATCCCTTGTCGCTGGTTTGCATTTC | Amplify *pduC* with BamHI restriction site for incorporation into pUC19 | This work |
| H143A | GAACACCATCACAACAGGCTGCCGTCACCAACATCAAAG | Site-directed mutagenesis primer | This work |

| | | | |
|---|---|---|---|
| E170A | CGGGTTTGATGAACAGGCAACCACTGTAGCCGTTG | Site-directed mutagenesis primer | This work |
| S362A | GCCAGGTACCGACTTTATCTCTGCCGGTTTTTCCG | Site-directed mutagenesis primer | This work |
| pduC* F | GGTAAAACAGTGCTTTCG | Amplification of *pduC** for recombination into genome | This work |
| pduC* R | CTTGTCGCTGGTTTGC | Amplification of *pduC** for recombination into genome | This work |
| RS-pduC 2 F | GGACATATGAGATCGAAAAGATTTGAAGC | Amplify *pduCDE* with NdeI restriction site for incorporation into pET30b | This work |
| RS-pduC 2 R | TCGAAGCTTTTAATCGTCGCCTTTCAG | Amplify *pduCDE* with HindIII restriction site for incorporation into pET30b | This work |

Oligonucleotides sequences used to generate the bacterial strains in this manuscript.