Supporting information

Supporting Methods

Bone marrow-derived dendritic cells. Bone marrow-derived dendritic cells (BMDCs) were generated from 8-15 week old B6 WT, *c-Rel*$^{-/-}$ and *p50*$^{-/-}$ mice. BM cells were harvested from femurs, and the red blood cells were lysed by using red blood cell lysis buffer. BM cells were cultured for 10 days in RPMI-1640 supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin, and 50μM β-mercaptoethanol. 20ng/mL of recombinant murine granulocyte macrophage colony-stimulating factor (Peprotech, Rocky Hill, NJ, United States) was added to cell cultures every two days. After 10 days of culture, the generated DCs were harvested. BMDCs ($3x10^5$ cell/well) were seeded in 96-well plates and cultured ± IL-36γ (100ng/mL) for 6 hours or 24 hours. For inhibition of NF-κB components, BMDCs was pretreated with the c-Rel inhibitor IT-603 (Calibiochem, San Diego, CA, United States) or p50 inhibitor peptide or p65 inhibitor peptide (Novus biological, Littleton, CO, United States) for 1 hour ± IL-36γ. After 1 hour of pre-treatment, media containing inhibitors and IL-36γ was replaced with new media and incubated for 6 hours to analyze gene expression or 24 hours to analyze protein expression.

Quantitative real-time PCR. Total RNA was isolated from colon tissues of mice using the Qiagen RNeasy Mini Kit and QIAcube with on-column DNase digestion according to the manufacturer's protocol. cDNA was generated using the Superscript First Strand Synthesis kit for RT-PCR and random hexamer primers (Invitrogen). Q-PCR was performed with SYBR Green using a StepOnePlus PCR system (Applied Biosystem) and gene expression was normalized to *gapdh*.

Chromatin Immunoprecipitation (ChIP) assay. BMDCs were cultured ± IL-36γ (100ng/mL) for 8 hours. ChIP assays were performed using EZ-ChIP kit (EMD Millipore 17371) according to manufacturer's protocol. Briefly, chromatin was obtained from the cultured cells after fixation with 18.5% formaldehyde and fragmented by sonication. One tenth of each fragmented chromatin sample was saved before immunoprecipitation for use as an input control. The remaining fragmented chromatin was immunoprecipitated with either control or anti-p50 (clone SC-8418) or anti-c-rel (clone sc-6955) antibodies (Santa Cruz Biotechnology). DNA recovered from the immunoprecipitation and input control were analyzed by qPCR.

Quantitation of fecal bacteria abundance. Fecal samples were collected before DSS treatment and 6 days after beginning DSS treatment. All feces were stored at -80°C until DNA extraction. The total genomic DNA from each fecal sample was extracted using the QIAamp DNA stool minikit according to the manufacturer's protocol. Samples were analyzed for bacterial gene expression using the qPCR assay with primers listed in Table 1.

Table 1

Il22	Fwd	5’-CAGGAGGTGGTGCCTTTCCT-3’
	Rev	5’-TGGTCGTCACCGCTGATGT-3’
Il23	Fwd	5’-GCTGTGCCTAGGAGTAGCAG-3’
	Rev	5’-TGGCTGTTGTCCTTGAGTCC-3’
Il1f6	Fwd	5’-TAGTGGGTGTAGTTCTGTAGTGTGC-3’
	Rev	5’-GTTCGTCTCAAGAGTGTCCAGATAT-3’
Il1f8	Fwd	5’-ACAAAAAGCCTTTCTGTTCTATCAT-3’
	Rev	5’-CCATGTTGGATTTACTTCTCAGACT-3’
Il1f9	Fwd	5’-AGAGTAACCCCAGTCAGCGTG-3’
	Rev	5’-AGGGTGGTGGTACAAATCCAA-3’
Il1rl2	Fwd	5’-AAACACCTAGCAAAAGCCCAG-3’
	Rev	5’-AGACTGCCCGATTTTCCTATG-3’
Reg3a	Fwd	5’-GGCACCGAGCCCAATG-3’
	Rev	5’-GGATTTCTCTCCCATGCAAAGT-3’
Reg3b	Fwd	5’-ATGCTGCTCTCCTGCCTGATG-3’
	Rev	5’-CTAATGCGTGCGGAGGGTATATTC-3’
Reg3g	Fwd	5’-TTCCTGTCCTCCATGATCAAAA-3’
	Rev	5’- CATCCACCTCTGTTGGGTTCA-3’
S100a8	Fwd	5’-TGAGTGTCCTCAGTTTGTGCAG-3’
	Rev	5’-TGTGAGATGCCACACCCACTTT-3’
S100a9	Fwd	5’-CAAATGGTGGAAGCACAGTTGGCA-3’
	Rev	5’-TTGTGTCCAGGTCCTCCATGATGT-3’
Gapdh	Fwd	5’-CAAATGGTGGAAGCACAGTTGGCA-3’
	Rev	5’-TTGTGTCCAGGTCCTCCATGATGT-3
Occludin	Fwd	5’-TTGAAAGTCCACCTCCTTACAGA-3’
	Rev	5’-CCGGATAAAAAGAGTACGCTGG-3’
Cldn2	Fwd	5'-GGCTGTTAGGCACATCCAT-3'
	Rev	5'-TGGCACCAACATAGGAACTC-3'
Clostridium XIVa	Fwd	5'-AAATGACGGTACCTGACTAA-3'
	Rev	5'-AAATGACGGTACCTGACTAA-3'
Clostridium XI	Fwd	5'-ACGGTACTTGAGGAGGA -3'
	Rev	5'-GAGCCGTAGCCTTTCACT -3'
Helicobacter	Fwd	5'-ACCAAGGC(A/ T)ATGACGGGTATC-3'

	Rev	5'-CGGAGTTAGCCGGTGCTTATT-3'
Desulfovibrio	Fwd	5'-CCGTAGATATCTGGAGGAACATCAG-3'
	Rev	5'-ACATCTAGCATCCATCGTTTACAGC-3'
Oscillibacter	Fwd	5'-ACGGTACCCCTTGAATAAGCC-3'
	Rev	5'-TCCCCGCACACCTAGTATTG-3'
Bacteroides	Fwd	5'-GAGAGGAAGGTCCCCCAC-3'
	Rev	5'-CGCTACTTGGCTGGTTCAG-3'
Prevotella	Fwd	5'-CACCAAGGCGACGATCA-3'
	Rev	5'-GGATAACGCCTGGACCT-3'
Alistipes	Fwd	5'-TTAGAGATGGGCATGCGTTGT-3'
	Rev	5'-TGAATCCTCCGTATT-3'
Lactobacillus	Fwd	5'-AGCAGTAGGGAATCTTCCA-3'
	Rev	5'-CACCGCTACACATGGAG-3'

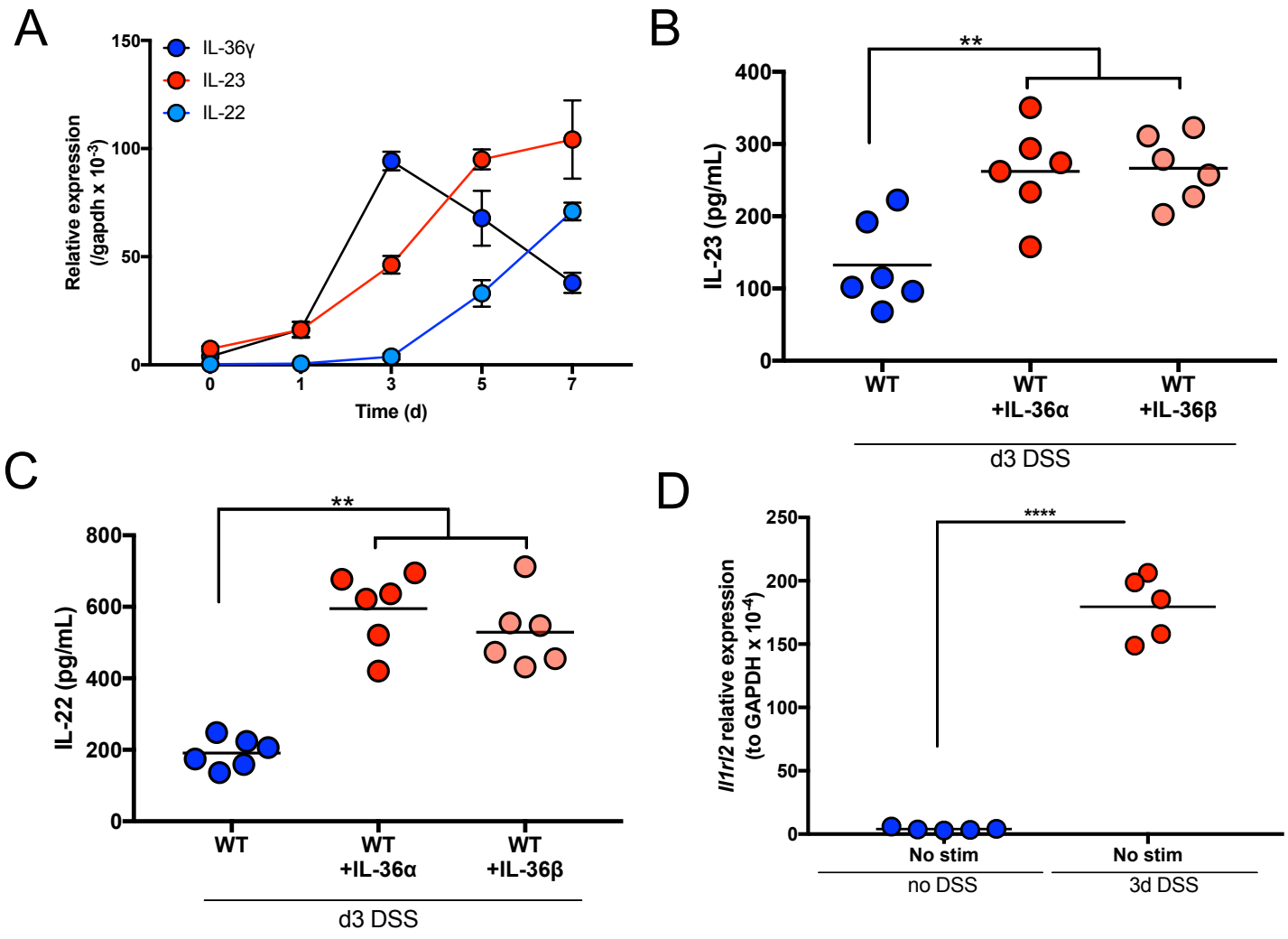

Fig. S1. Expression of IL-36γ, IL-23, IL-22 and IL-36R during DSS treatment. *(A)* Time course analysis of IL-36g, IL-23, and IL-22 mRNA expression in colonic tissue isolated from WT mice during the course of DSS treatment (5 days DSS followed by 2 days of regular water). *(B-C)* Colonic explants from DSS-treated mice at day 3 DSS stimulated with IL-36α and IL-36β for 60h and supernatants were analyzed for IL-23 *(B)* and IL-22 *(C)* by ELISA. *(D)* qPCR analysis of *Il1rl2* expression in colonic explants isolated from DSS-treated mice as in *(B-C)*. Data are representative of at least 2 independent experiments with 4-5 mice/group. All data are presented as mean ± SEM; $^{**}P < 0.01$ and $^{****}P < 0.0001$, Student's unpaired *t* test.

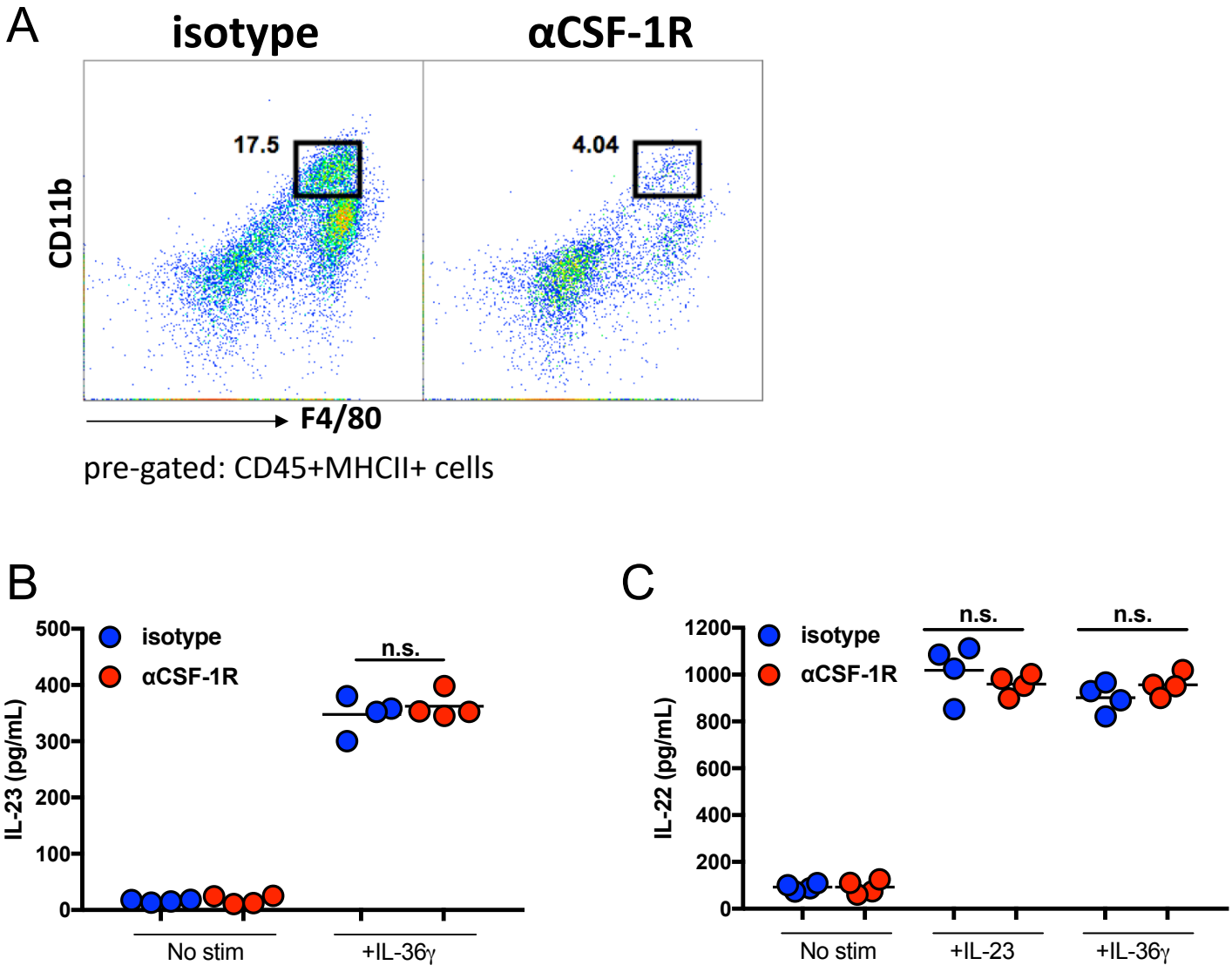

Fig. S2. Injection of anti-CSF-1R antibody does not alter IL-36γ-induced IL-23 or IL-22 production. *(A)* FACS plots of total colonic lamina propria cells isolated from WT mice treated with neutralizing anti-CSF-1R antibody or isotype control antibody and analyzed at day 3 of DSS treatment. *(B-C)* Colonic explants from DSS-treated mice as in *(A)* were cultured for 60h in the presence or absence of IL-36γ or IL-23. Supernatants were analyzed for IL-23 *(B)* and IL-22 *(C)* expression by ELISA. All data are presented as mean ± SEM; n.s.= not significant; one-way ANOVA with Tukey's multiple comparison test.

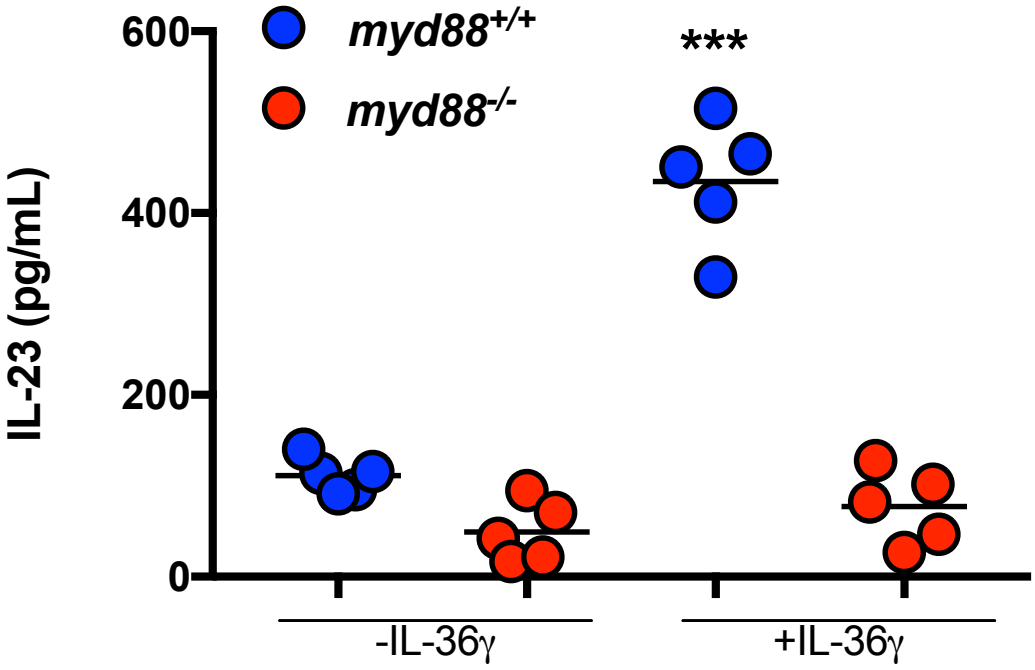

Fig. S3. IL-36γ signaling stimulates IL-23 production in BMDCs via MyD88. WT (*myd88*$^{+/+}$) or *myd88*$^{-/-}$ BMDCs were cultured for 60h in the presence or absence of IL-36γ. Supernatants were analyzed for IL-23 by ELISA. Data are representative of 2 independent experiments with 4-5 mice/group. All data are presented as mean ± SEM; ***$P < 0.001$, one-way ANOVA with Tukey's multiple comparison test.

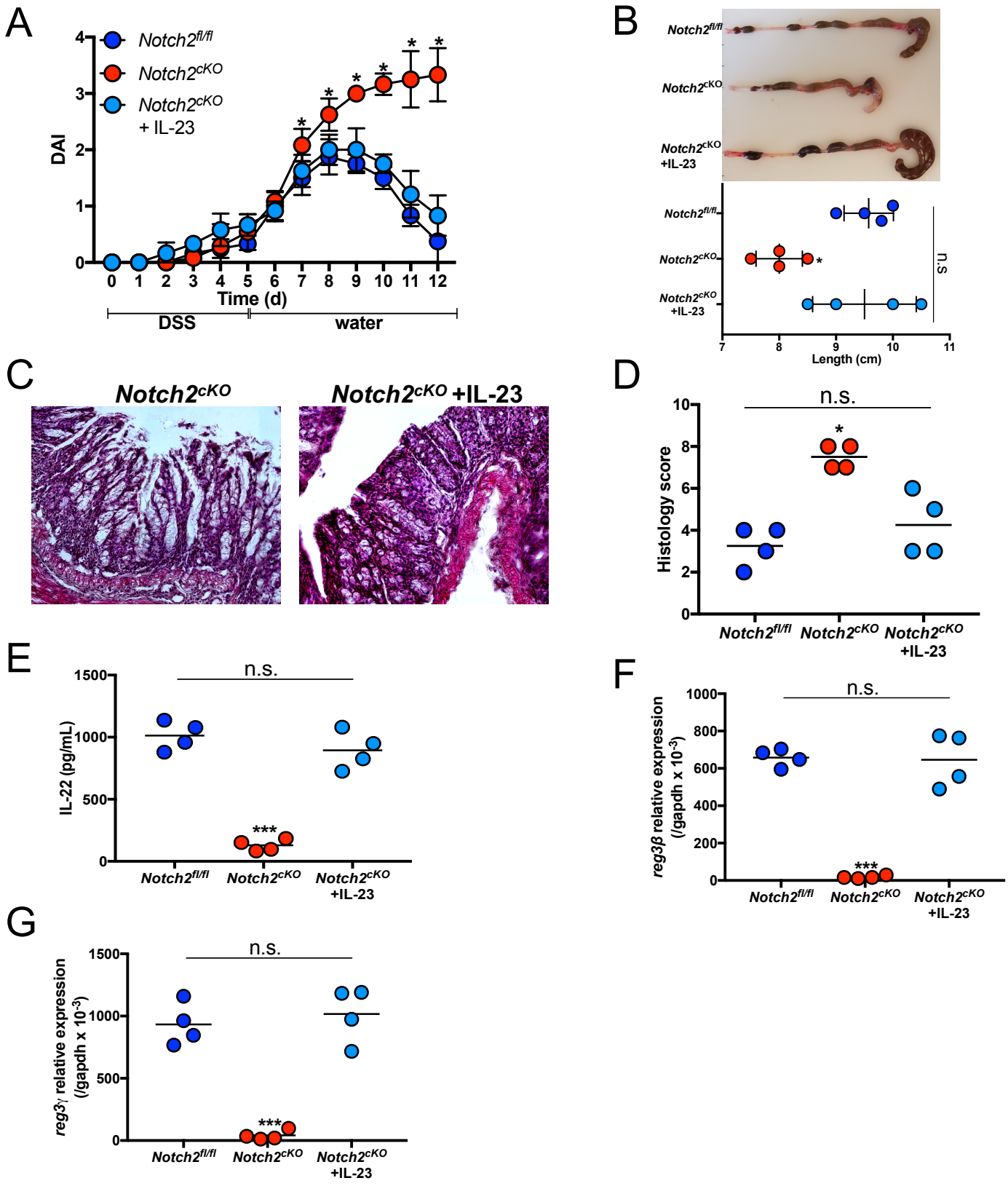

Fig S5. Systemic IL-23 administration induces the resolution of DSS-induced colonic damage in *Notch2*^{cKO} mice. *(A)* DAI of *Notch*$^{fl/fl}$ and *Notch2*^{cKO} mice treated with DSS for 5 days, followed by normal water for 7days, in the presence or absence of IL-23. *(B)* Image and length of colons from mice treated as in *(A)*. *(C)* H&E staining and *(D)* histology scoring of colon sections from mice treated as in *(A)*. *(E)* IL-22 protein expression in colons from *Notch*$^{fl/fl}$ and *Notch2*^{cKO} mice treated with DSS for 5 days in the presence or absence of IL-23. *(F)* Reg3β and *(G)* Reg3γ mRNA expression in colons isolated from mice as in *(A)*. Data are representative of 3 independent experiments with 4-5 mice/group. All data are presented as mean ± SEM; n.s.= not significant; *$P < 0.05$ and ***$P < 0.001$, one-way ANOVA with Tukey's multiple comparison test.

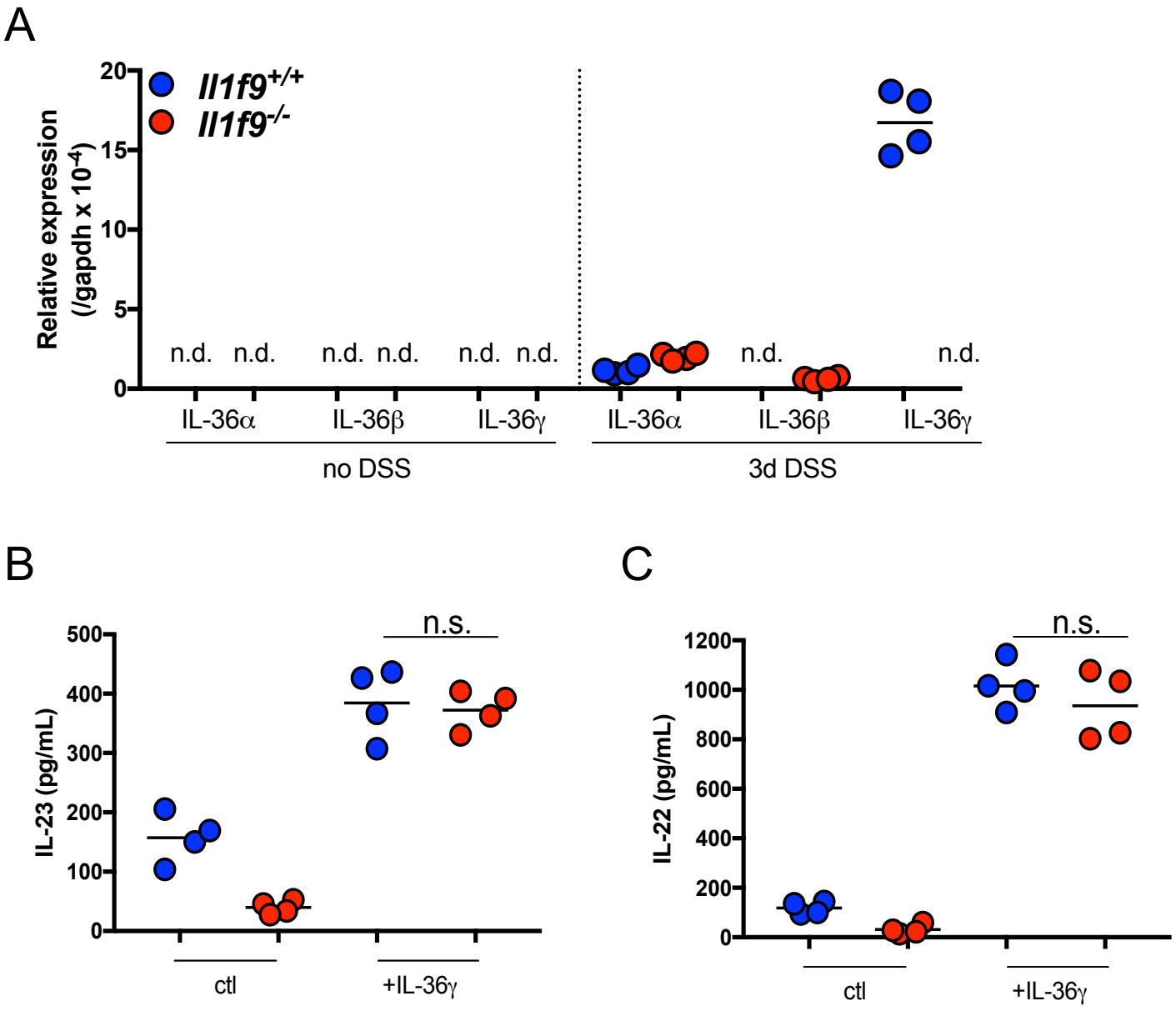

Fig. S5. Analysis of *Il1f9*$^{-/-}$ mice. *(A)* IL-36 ligand mRNA expression in colonic tissues from 3-day DSS-treated *Il1f9*$^{+/+}$ and *Il1f9*$^{-/-}$ mice. *(B)* Colonic explants from control (no DSS) or 3-day DSS-treated (3d DSS) *Il1f9*$^{+/+}$ and *Il1f9*$^{-/-}$ mice were cultured for 60h in the presence or absence of IL-36*γ*. Supernatants were analyzed for IL-23 *(B)* and IL-22 *(C)* by ELISA. n.d.= not detected. Data are representative of 2 independent experiments with 3-4 mice/group. All data are presented as mean ± SEM; n.s.= not significant.

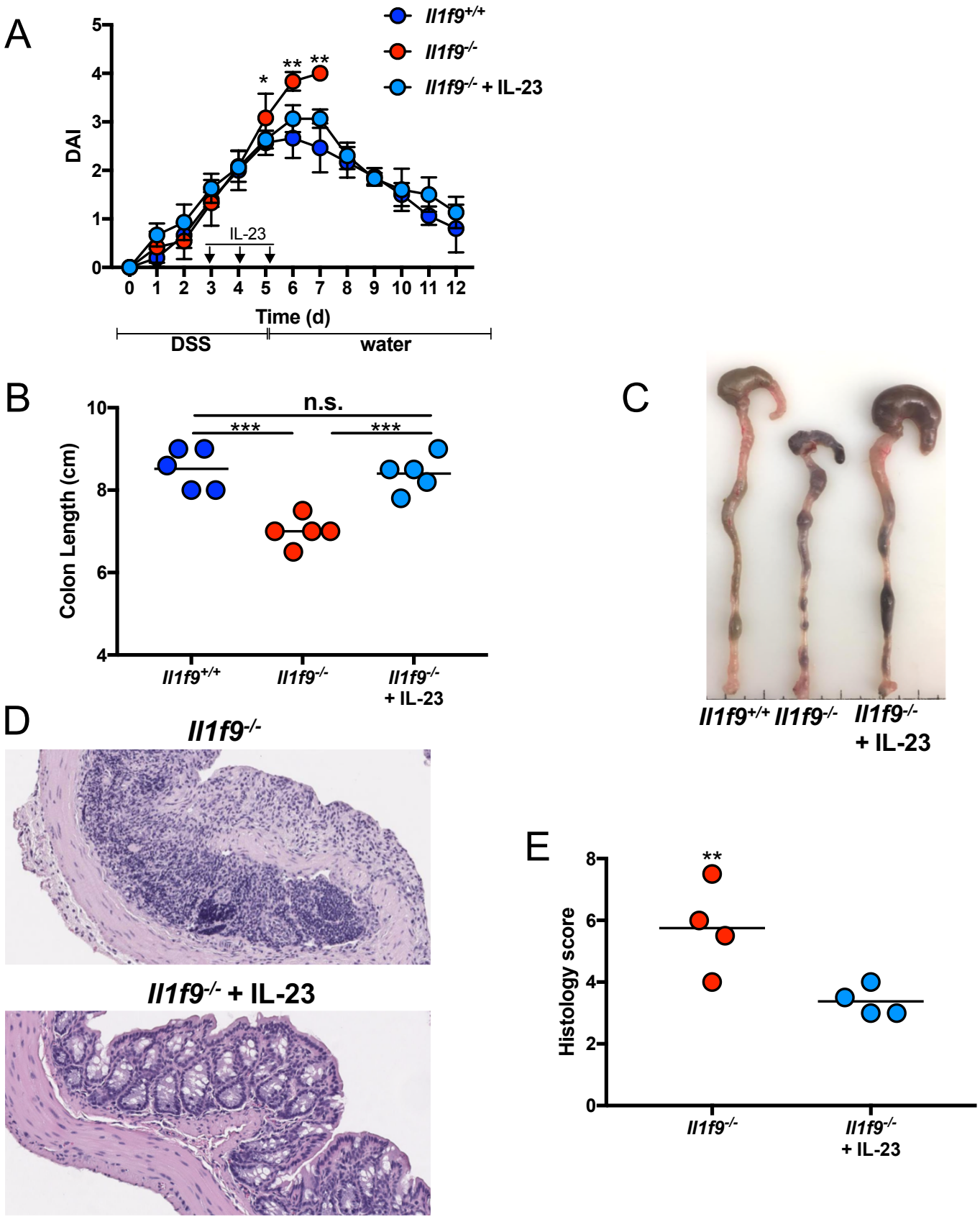

Fig. S6. Systemic IL-23 administration induces resolution of DSS-induced colonic damage in *Il1f9*$^{-/-}$mice. *(A)* DAI of *Il1f9*$^{+/+}$ and *Il1f9*$^{-/-}$ mice treated with DSS for 5 days, followed by normal water for 7days, in the presence or absence of IL-23. *(B-C)* Image and length of colons from mice treated as in *(A)*. *(D)* H&E staining and *(E)* histology scoring of colon sections from mice treated as in *(A)*. Data are representative of 3 independent experiments with 4-5 mice/group. All data are presented as mean ± SEM; n.s.= not significant; $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$, one-way ANOVA with Tukey's multiple comparison test.

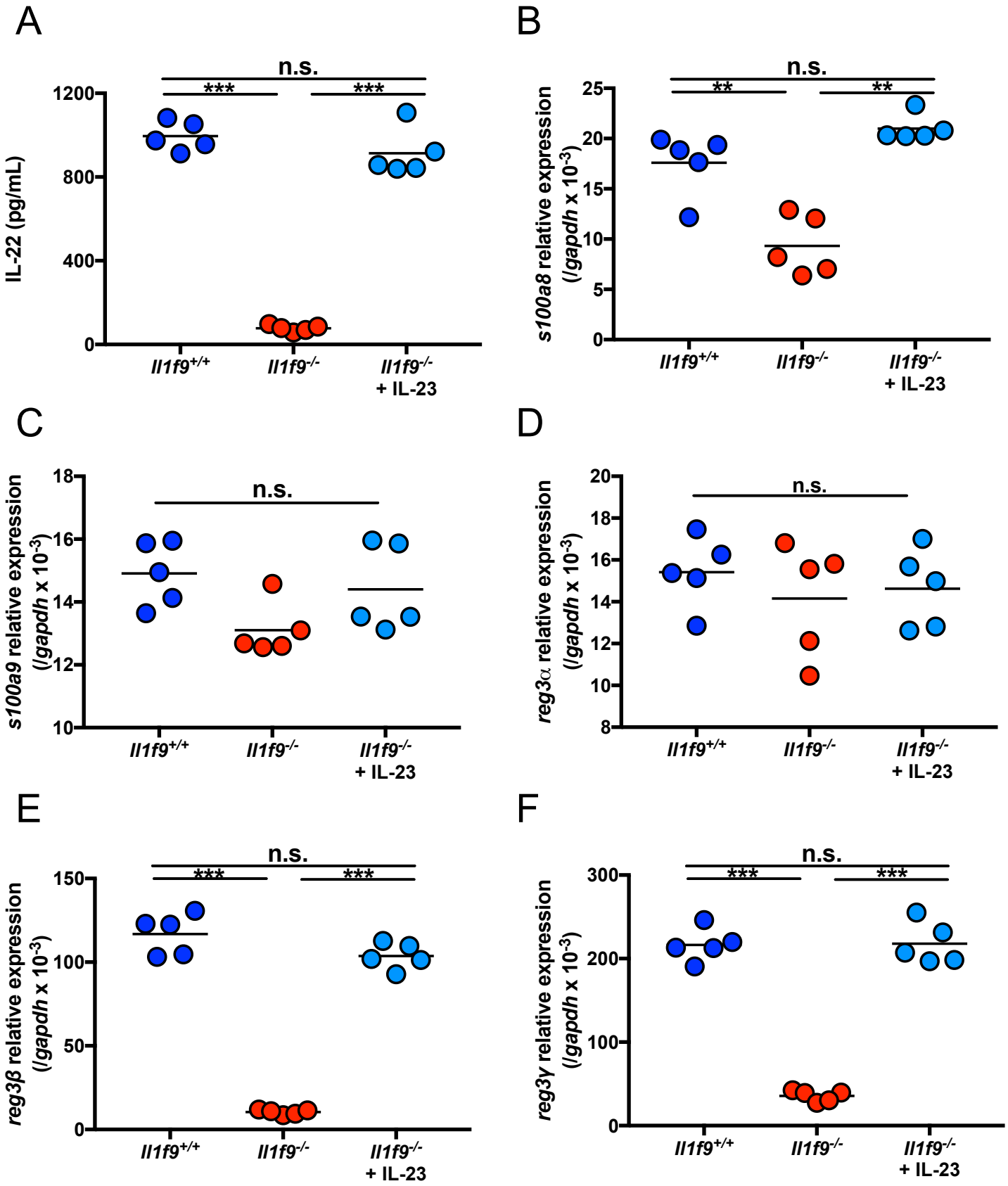

Fig. S7. Systemic IL-23 administration induces IL-22 and AMPs in DSS-treated *Il1f9*$^{-/-}$ mice. *(A)* IL-22 protein expression in colons from *Il1f9*$^{+/+}$ and *Il1f9*$^{-/-}$ mice treated with DSS for 5 days in the presence or absence of IL-23. *(B)* S100A8 **(***C***)** S100A9 *(D)* Reg3α *(E)* Reg3β and *(F)* Reg3*γ* mRNA expression in colons isolated from mice as in *(A)*. Data are representative of 2 independent experiments with 5-6 mice/group. All data are presented as mean ± SEM; n.s.= not significant; $^{**}P < 0.01$, and $^{***}P < 0.001$, one-way ANOVA with Tukey's multiple comparison test.

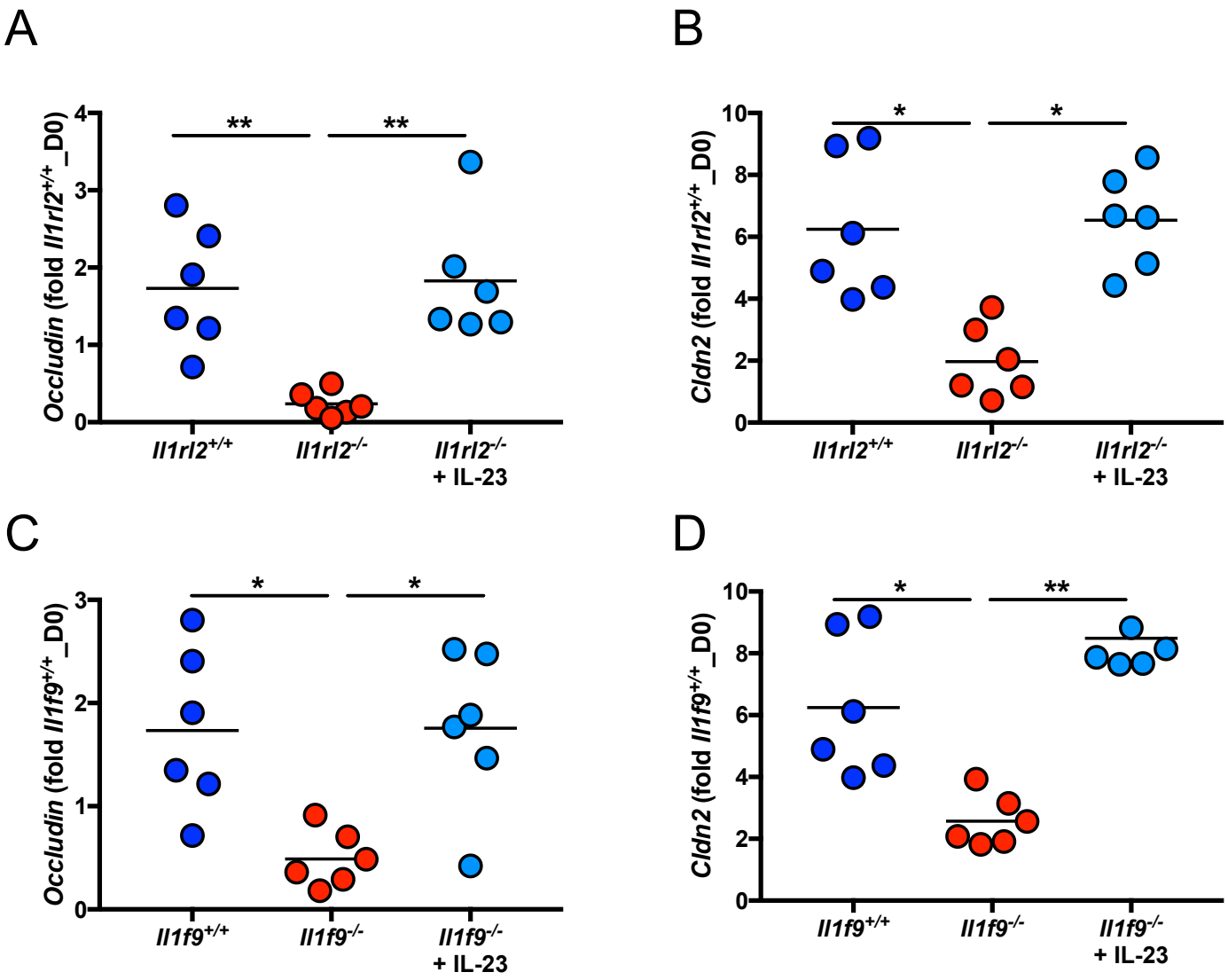

Fig. S8. Systemic IL-23 administration induces expression of occludin and claudin 2 in DSS-treated *Il1rl2*$^{-/-}$ and *Il1f9*$^{-/-}$ mice. *(A)* Occludin and *(B)* claudin 2 mRNA expression in colons from *Il1rl2*$^{+/+}$ and *Il1rl2*$^{-/-}$ mice treated with DSS for 5 days in the presence or absence of IL-23. *(C)* Occludin and *(D)* claudin 2 mRNA expression in colons from *Il1f9*$^{+/+}$ and *Il1f9*$^{-/-}$ mice treated with DSS for 5 days in the presence or absence of IL-23. Data are combined from 2 independent experiments with 3 mice/group. All data are presented as mean ± SEM; **P* < 0.05 and ***P* < 0.01, one-way ANOVA with Tukey's multiple comparison test.

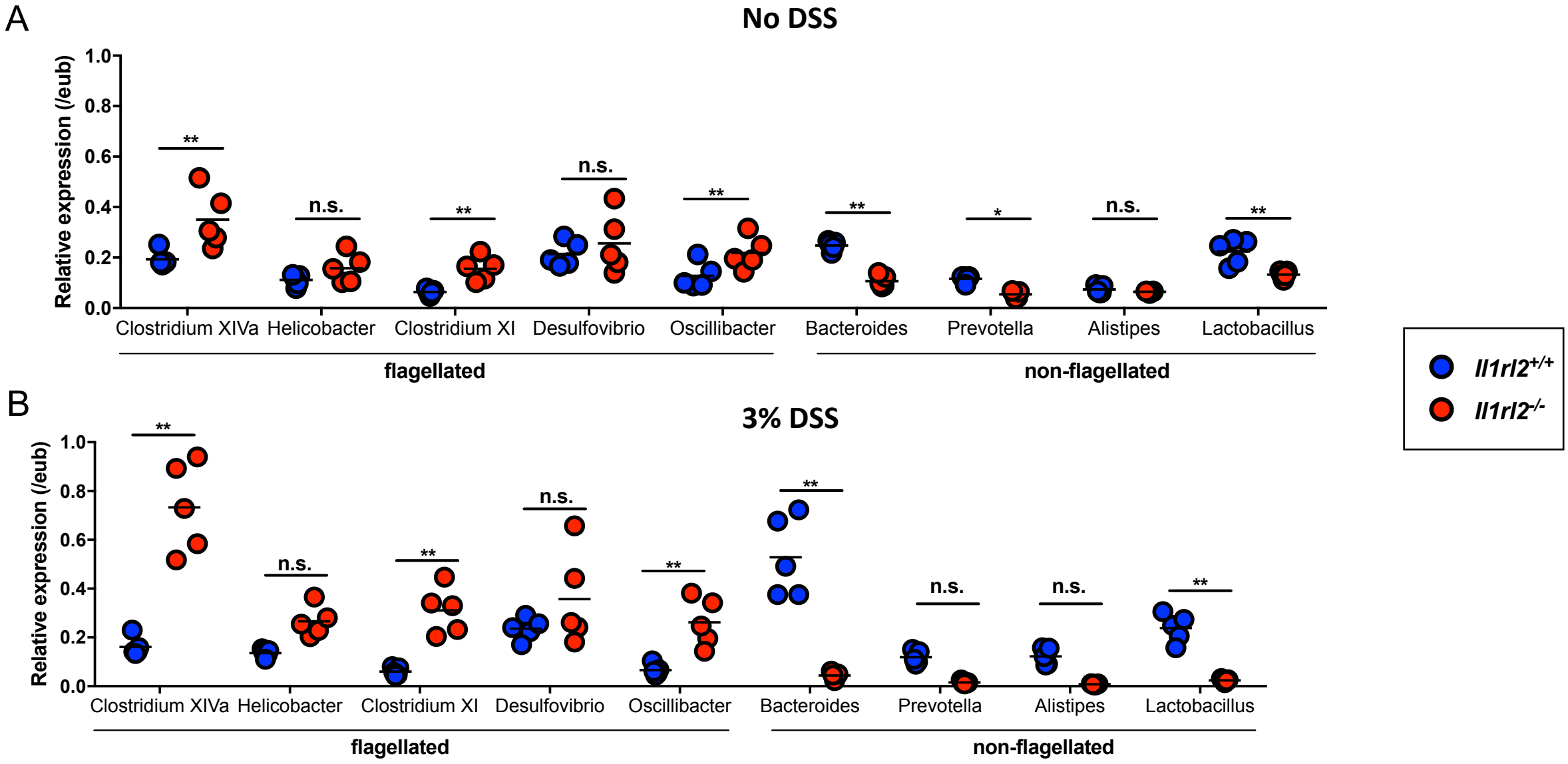

Fig. S9. Fecal microbiota composition in steady-state and DSS-treated *Il1rl2*$^{-/-}$ mice. Real-time PCR analysis of flagellated and non-flagellated bacterial groups in the feces of *Il1rl2*$^{+/+}$ mice *and Il1rl2*$^{-/-}$ *(A)* before DSS treatment (no DSS) and *(B)* after DSS treatment. All data are presented as mean ± SEM; **P* < 0.05 and ***P* < 0.01, one-way ANOVA with Tukey's multiple comparison test.

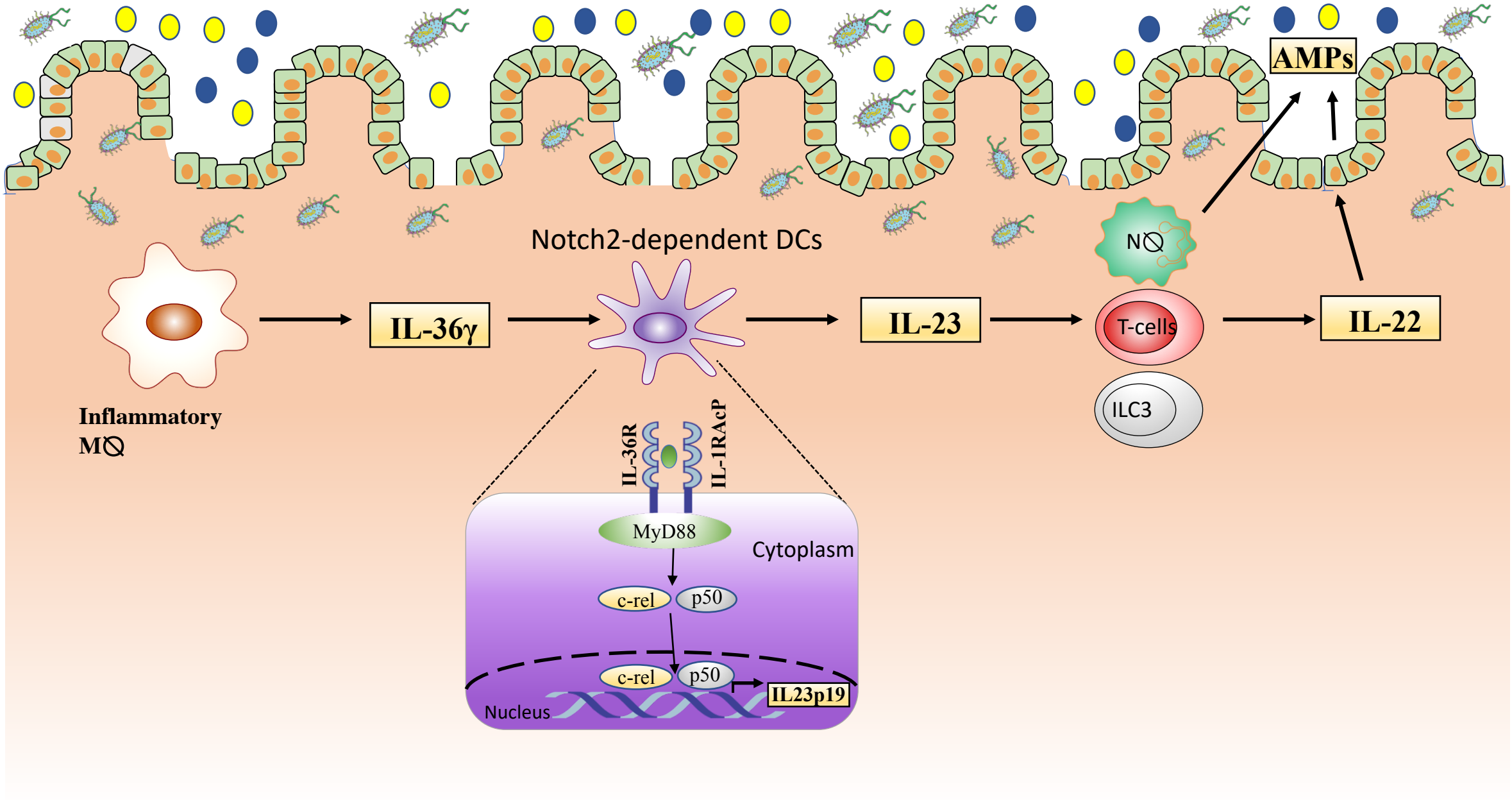

Fig. S10. Schematic representation of the IL-36/IL-23/IL-22/AMPs axis.