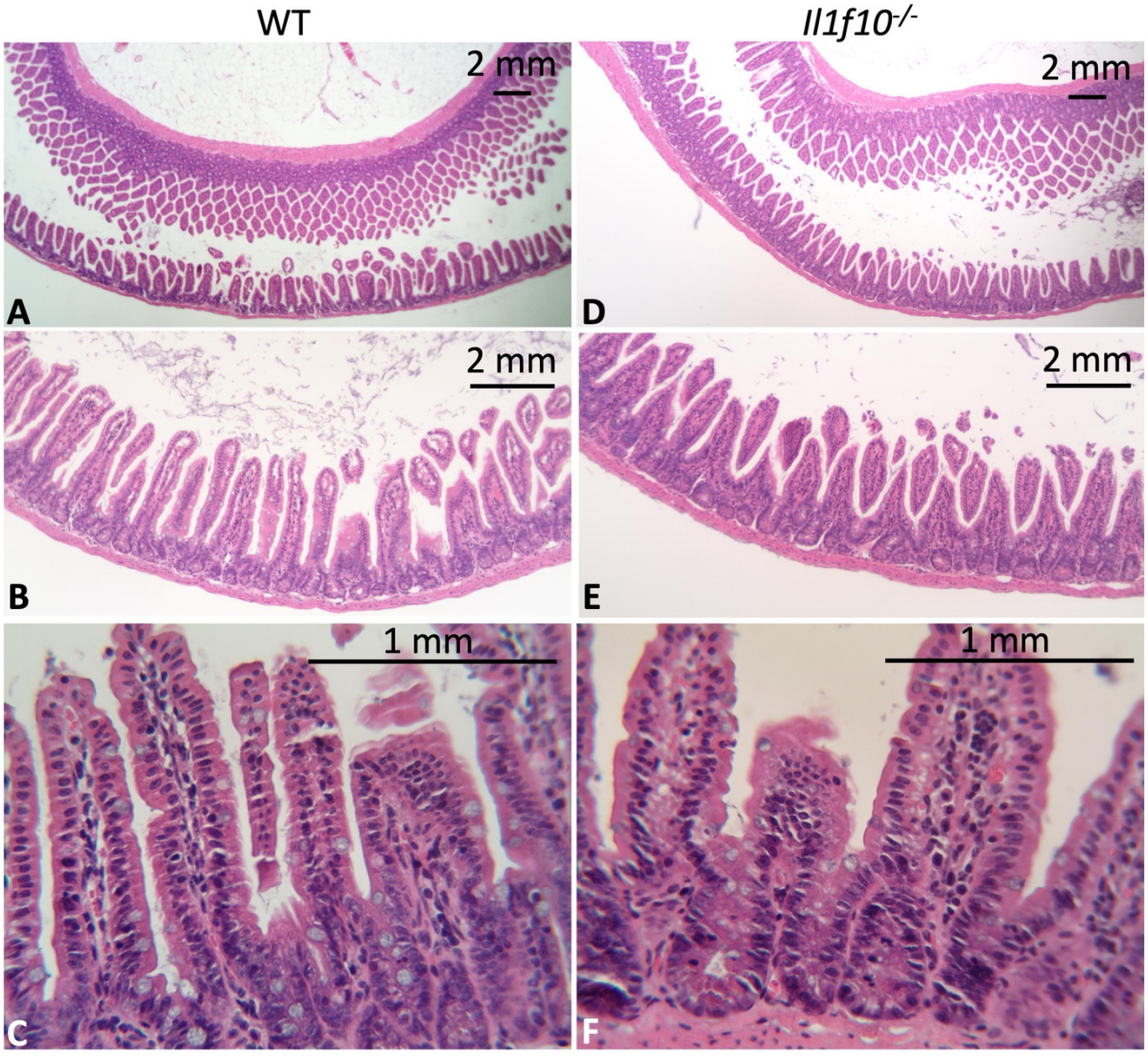
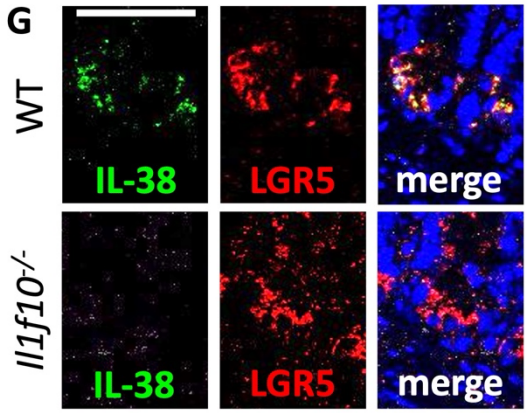
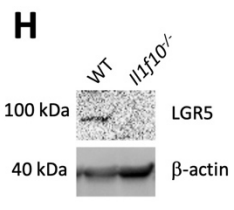
Supplementary







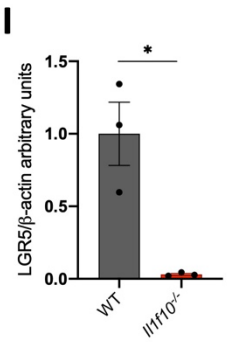


Figure S1: **Characterization of *Il1f10* deficient SI.** (**A-F**) Histological analysis of SI of wild type and *Il1f10*$^{-/-}$ mice. (**G**) Staining of IL-38 (green) and LGR5 (red) in wild type and *Il1f10*$^{-/-}$ SI. Scale bar = 50 μm. (**H**) A representative western blot performed on wild type and *Il1f10* knockout intestines. (**I**) Histogram derived from western blot shown in (H) of LGR5 levels in wild type and *Il1f10* knockout intestines. n = 3 biological replicates for each genotype. Comparisons between wild type and *Il1f10* knockout samples were performed with Student *t*-test. * $p < 0.05$. Mean ± SEM.

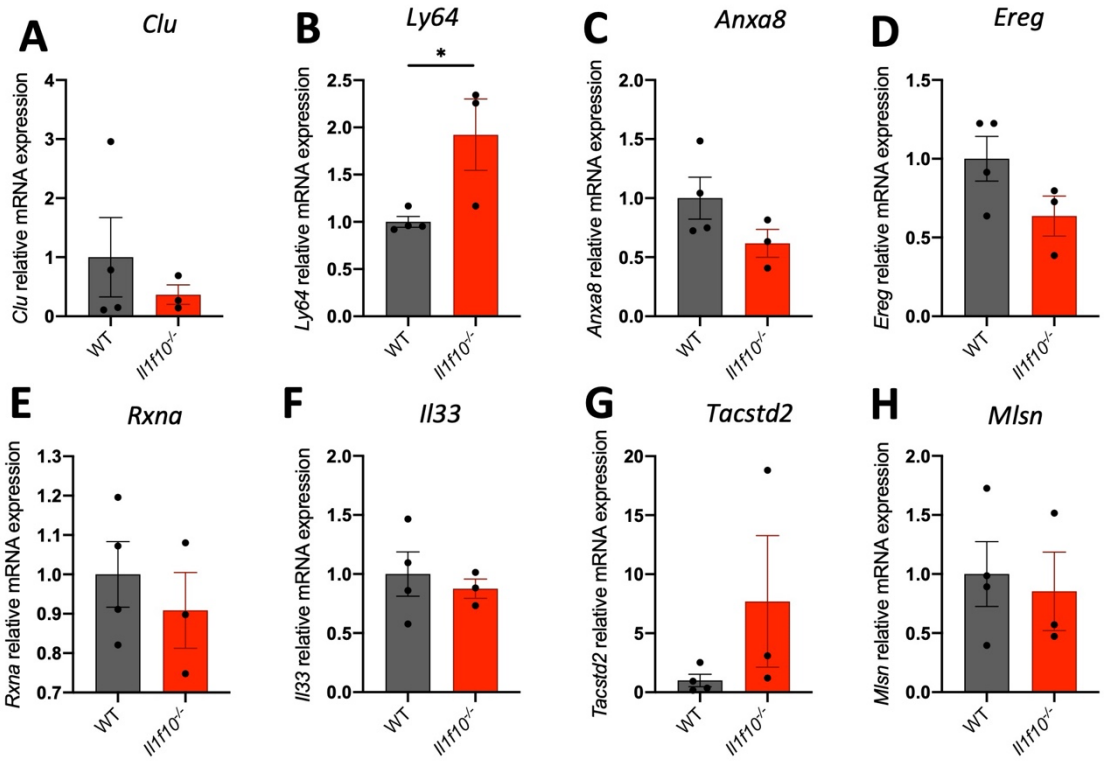


Figure S2: **Transcriptional analysis of regeneration-related genes.** (**A-H**) Gene expression analysis of *Clu* (**A**), *Ly64* (**B**), *Anxa8* (**C**), *Ereg* (**D**), *Rxna* (**E**), *Il33* (**F**), *Tacstd2* (**G**) and *Mlsn* (**H**) in SI samples from wild type and *Il1f10* deficient mice. Comparisons between wild type and *Il1f10* knockout samples were performed with Student *t*-test. * $p < 0.05$. Mean ± SEM.

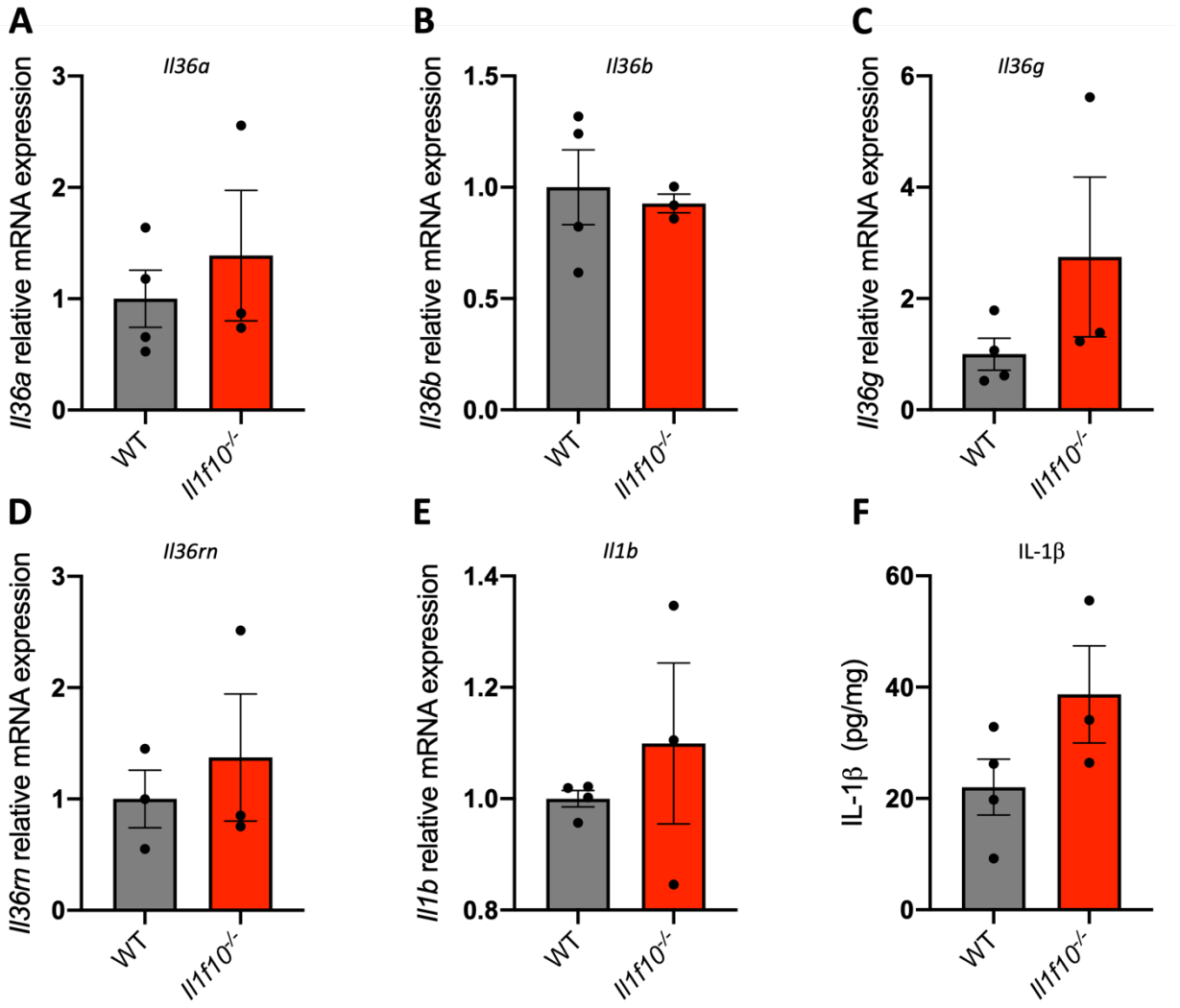


Figure S3: **Interleukins expression is not significantly altered in *Il1f10* deficient intestines.** (**A-E**) Gene expression analysis of *Il36a* (**A**), *Il36b* (**B**), *Il36g* (**C**), *Il36rn* (**D**) and *Il1b* (**E**) in SI samples from wild type and *Il1f10* deficient mice. Comparisons between wild type and *Il1f10* knockout samples were performed with Student *t*-test. (**F**) Intracellular IL-1β expression in the SI lysates from wild type and *Il1f10* deficient mice. Comparisons between wild type and *Il1f10* knockout samples were performed with Student *t*-test. Mean ± SEM.

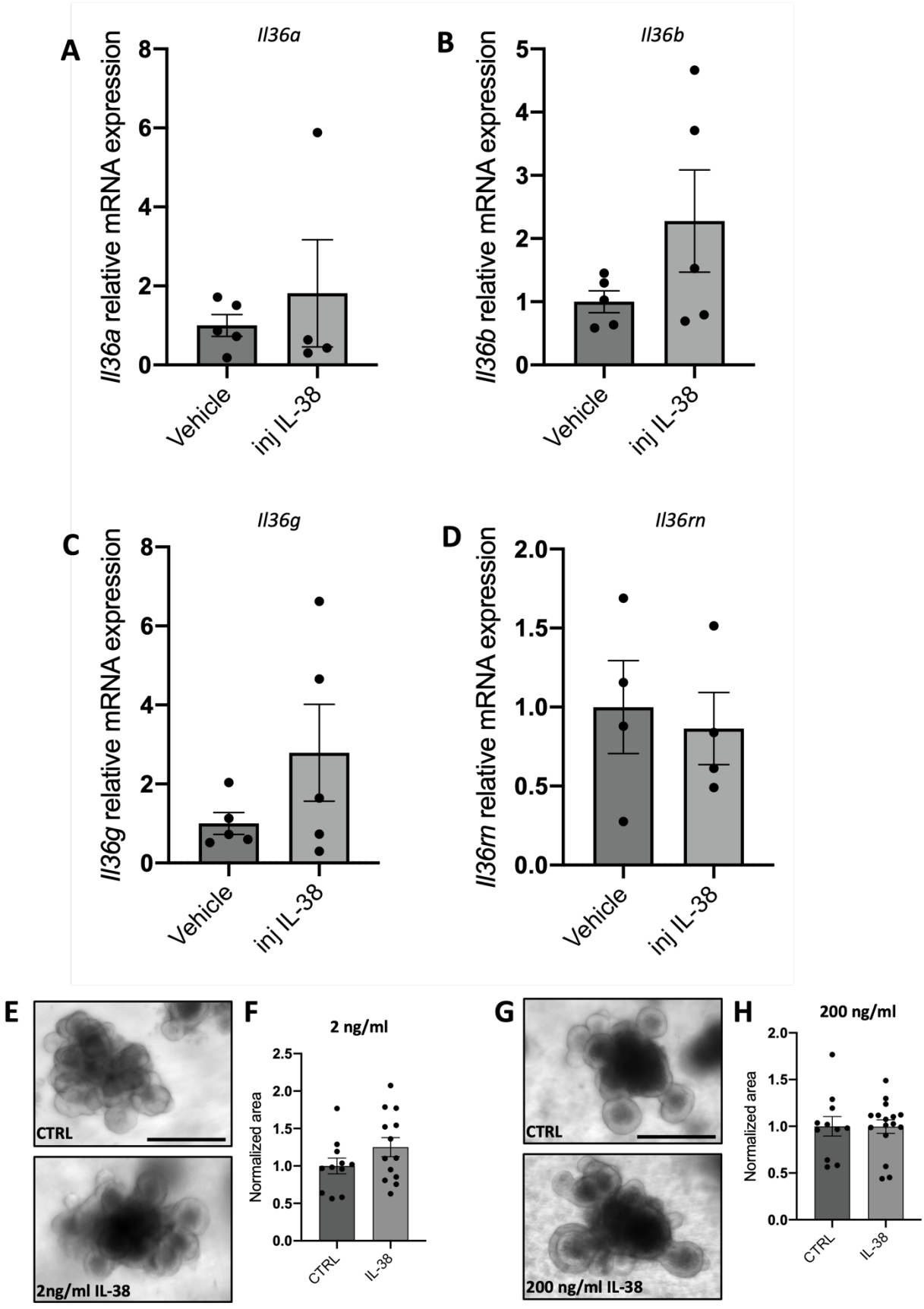

A
Il36a
Il36a relative mRNA expression
8
6
4
2
0
Vehicle
inj IL-38
B
Il36b
Il36b relative mRNA expression
5
4
3
2
1
0
Vehicle
inj IL-38
C
Il36g
Il36g relative mRNA expression
8
6
4
2
0
Vehicle
inj IL-38
D
Il36rn
Il36rn relative mRNA expression
2.0
1.5
1.0
0.5
0.0
Vehicle
inj IL-38
E
CTRL
2ng/ml IL-38
F
2 ng/ml
Normalized area
2.5
2.0
1.5
1.0
0.5
0.0
CTRL
IL-38
G
CTRL
200 ng/ml IL-38
H
200 ng/ml
Normalized area
2.0
1.5
1.0
0.5
0.0
CTRL
IL-38

Figure S4: **Tests of IL-38 effects *in vivo* and *ex vivo*.** (**A-D**) Gene expression analysis of *Il36a* (**A**), *Il36b* (**B**), *Il36g* (**C**), and *Il36rn* (**D**) in SI from mice injected with PBS (Vehicle) and with 1 μg recombinant IL-38 (inj IL-38) every day for 15 days. Comparisons between wild type and *Il1f10* knockout samples were performed with Student *t*-test. Mean ± SEM. (**E**) Representative pictures and measurements (**F**) of organoids treated with vehicle and 2 ng/ml IL-38. Each dot represents an individual organoid, organoid cultures were produced from 3 different mice and plated in three wells for each experimental condition. Scale bar: 200 μm. Comparisons between vehicle- and 2 ng/ml IL-38-treated organoids were performed with Student's *t*-test. (**G**) Representative pictures and measurements (**H**) of organoids treated with vehicle and 200 ng/ml IL-38. Each dot represents an individual organoid, organoid cultures were produced from 3 different mice and plated in three wells for each experimental condition. Scale bar: 200 μm. Comparisons between vehicle- and 200 ng/ml IL-38-treated organoids were performed with Student's *t*-test. Mean ± SEM.

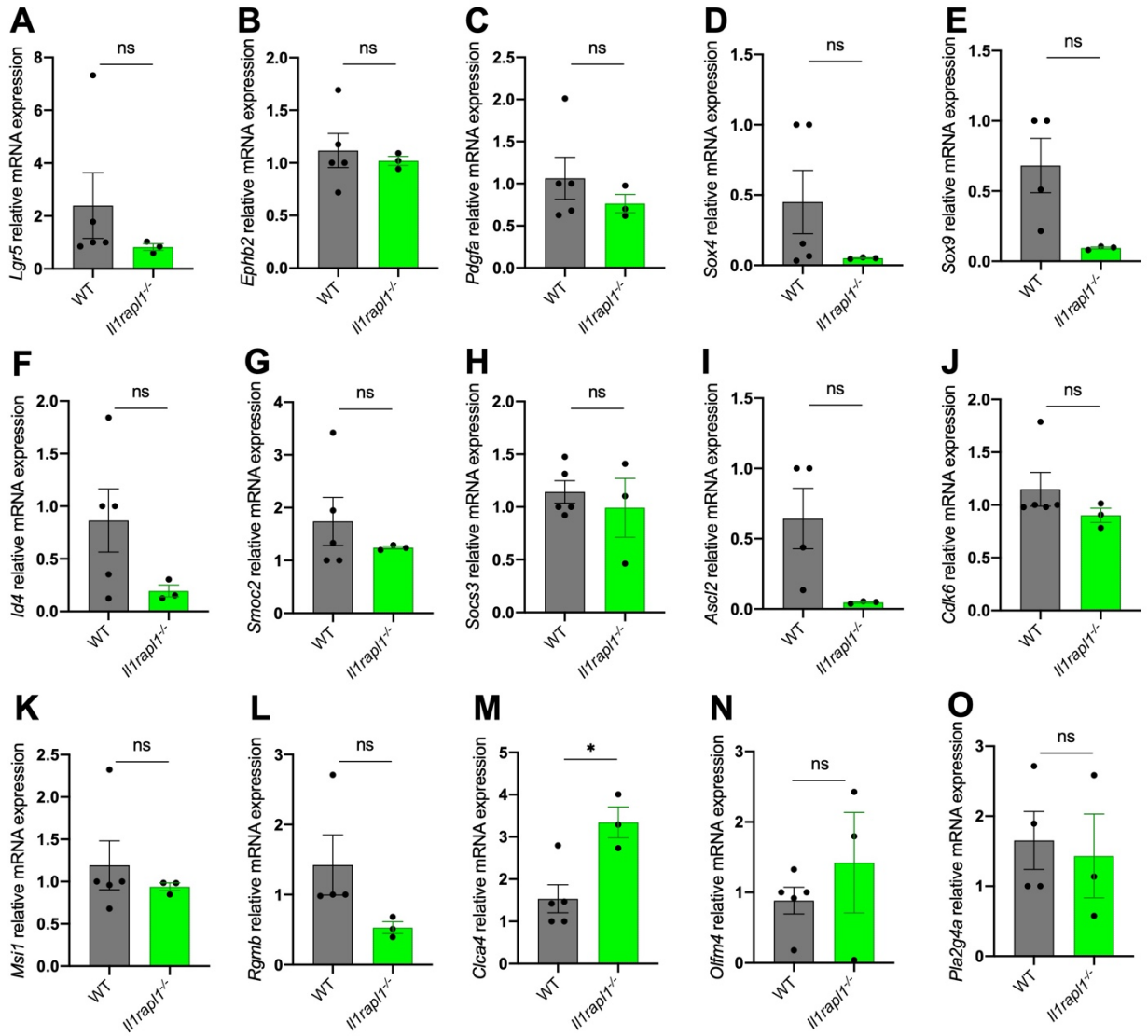


Figure S5: **Crypt markers in IL-1R9 KO intestines**. (**A-O**) Gene expression analysis of *Lgr5* (**A**), *Ephb2* (**B**), *Pdgfa* (**C**), *Sox4* (**D**), *Sox9* (**E**), *Id4* (**F**), *Smoc2* (**G**), *Socs3* (**H**), *Ascl2* (**I**), *Cdk6* (**J**), *Msi1* (**K**), *Rgmb* (**L**), *Clca4* (**M**), *Olfm4* (**N**) and *Pla2g4a* (**O**) in intestines from wild type and *Il1rapl1* deficient mice. Comparisons between wild type and *Il1rapl1* deficient intestines were performed with two-tailed unpaired Student's *t*-test. Wild type controls are the same used in Figure 1. * $p < 0.05$, ns = not significant. Mean ± SEM.

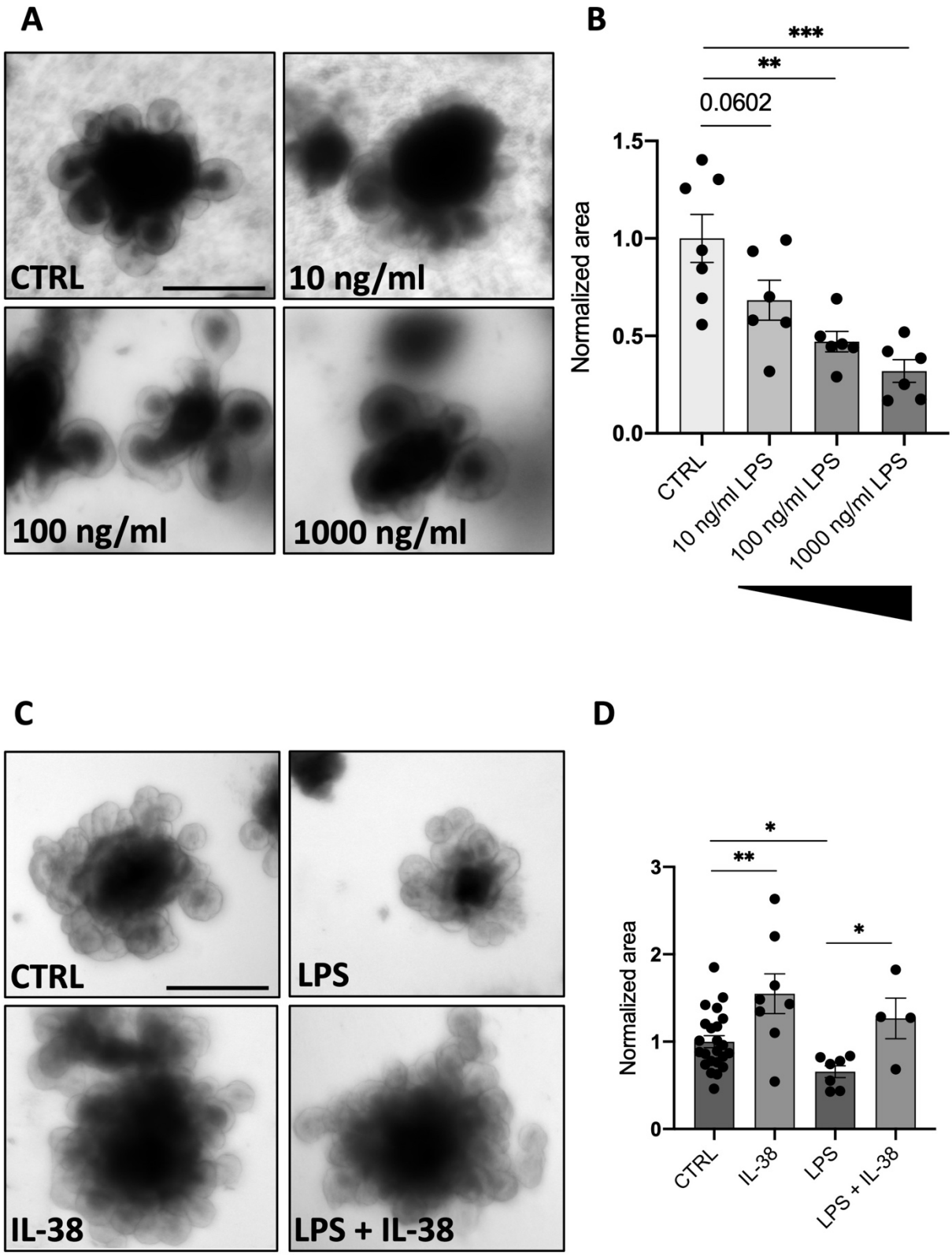


Figure S6: **LPS has negative effects on organoid growth.** (**A**) Representative pictures and measurements (**B**) of wild type organoids treated with vehicle, 10 ng/ml, 100 ng/ml and 1000

ng/ml LPS for 6 days. Each dot represents an individual organoid, organoid cultures were produced from 3 different mice and plated in three wells for each experimental condition. Scale bar: 200 μm. Comparisons between the four different experimental groups were performed with One-way ANOVA. (**C**) Representative pictures and measurements (**D**) of wild type organoids treated with vehicle, 20 ng/ml IL-38, 100 ng/ml LPS, 20 ng/ml IL-38 + 100 ng/ml LPS for 6 days. Each dot represents an individual organoid, organoid cultures were produced from 3 different mice and plated in three wells for each experimental condition. Comparisons were performed with two-tailed unpaired Student *t*-test. Scale bar: 200 μm. *p < 0.05; **p < 0.01; ***p < 0.001. Mean ± SEM.

Gene	Forward primer	Reverse primer	Reference
Lgr5	GATGCTGCTCAGGGTGGA	CGGGTAGCTGACTGATGTTG	This paper
Fzd5	CTGTGCTGTGCTTCATCTCC	CAGGGCCGGTAGTCTCATAG	This paper
Ephb2	TCTATGTCTTCCAGGTGCGG	TTCCTTGATGCTGGTCTGGT	This paper
Bmpr1a	ATGCTCCATGGCACTGGTATGA	GGCAGTGTCCTGAGCAATAGCA	Qi *et al.,* 2017
Id1	ATCGCATCTTGTGTCGCTGAG	AGTCTCTGGAGGCTGAAAGGT	Qi *et al.,* 2017
Olfm4	CGAGACTATCGGATTCGCTATG	TTGTAGGCAGCCAGAGGGAG	Qi *et al.,* 2017
Ascl2	GCCTGACCAAATGCCAAGTG	ATTTCCAAGTCCTGATGCTGC	Qi *et al.,* 2017
Rgmb	CACGGATCAGAAGGTGTACCA	AGCTGTCGCACAAACACTGT	Qi *et al.,* 2017
Pdgfa	GTAACACCAGCAGCGTCAAGT	TGGCTTCTTCCTGACATACTCC	Qi *et al.,* 2017
Sox9	GCCAGATGGACCCACCAGTAT	TCCAAACAGGCAGGGAGATTC	Qi *et al.,* 2017
Cdk6	GGCGTACCCACAGAAACCATA	AGGTAAGGGCCATCTGAAAACT	Qi *et al.,* 2017
Cdca7	ATGTCATCAGTGTCGCCAGAAA	CCTCGCCATAGCGGTTTCG	Qi *et al.,* 2017
Pla2g4a	GCATTTCTTGATGGGTGTCTGG	AGCCTCATCATCACTGTCGGA	Qi *et al.,* 2017
Sox4	ATGAACGCCTTTATGGTGTGG	TCCTGGATGAACGGAATCTTGT	Qi *et al.,* 2017
Smoc2	CCCAAGCTCCCCTCAGAAG	GCCACACACCTGGACACAT	Qi *et al.,* 2017
Clca4	GAAAGAACACTGTCGAAGCAAC	CAGTCTGGGATTTGTTTGGGATA	Qi *et al.,* 2017
Msi1	CCAGGGTTCCAAGCCACGA	CCATAAGCCGTGAGAGGGATA	Qi *et al.,* 2017
Phlda	GTGGTGATGACGGAGGGCA	TGCTTCTGCCTGGTAGACTTG	Qi *et al.,* 2017
Socs3	ATTTCGCTTCGGGACTAGC	AACTTGCTGTGGGTGACCAT	Carbognin *et al*., 2016
Si	GGGTCCAGCTTTTATGGTGA	TATGTGTTCTGTGCCGGTTC	Zhong *et al.,* 2015
ChgA	CCA ATA CCC AAT CAC CAA CC	TTGTAGCCTGCATGGAAGTG	Zhong *et al.,* 2015
Tff3	GTCCAAGGGTAGCAAGCATC	CTTGTGTTGGCTGTGAGGTC	Zhong *et al.,* 2015
Lyz1	CAGCTCACTAGTCGCTCCTG	TAGAGCTGCCCCTTTCATCT	Zhong *et al.,* 2015
Il1rl2	CCTTTCACGTGTCATGCTGG	CAACCAGAAGTAGGAACGCC	This paper
Clu	GTGAAGATGACCGCACTGTG	AGCTCCTTGTACTGCTCTGT	This paper
Ly64	GCTGATTCTTCTTGTGGCCC	CCACAATAACTGCTGCCTCC	This paper
Anxa8	AGCGTGAAGGGTAGTTCTCA	CTTGAAGGACTTGGCAATCTG	This paper
Ereg	GCAGTTATCAGCACAACCGT	TCTCTCTCATGTCCACCAGG	This paper
Rxna	GTCCGCCCTTCTCTGTCAT	CTAGTGGCGGCTTGATATCC	This paper
Il33	GATTTCCCCGGCAAAGTTCA	TGCAGTAGACATGGCAGAATTC	This paper
Tacstd2	GACAACGATGGCCTCTACGA	CAGCTCGGAGTCTAGGTCAG	This paper
Mlsn	ATCGAAGTGGTCAGTCTCCA	CCTTGGGTGTATGACGGTCA	This paper
Il36rn	TGCACAATAACCAGCTGCTG	CACTGGCTTCCTCCTTGAAC	This paper
Il36a	ACTCTTGAGACGAACAGGGG	AGAGAGGCTTTTACAGGTTCCT	This paper
Il36b	ACAGATGGTATGGGTCCTGAC	TTTCTGTTCTTGATTCCCAGGA	This paper
Il36g	ACCCAGAGTCTCTTGAACAGG	GTCCGGGTGTGGTAAAACAG	This paper
Il1b	CAACCAACAAGTGATATTCTCCATG	GATCCACACTCTCCAGCTGCA	Seo *et al*., 2015
Il1f10	AGGATGCACATCAAAAGGCTT	GGCCTCGGTTAGGAAGGATAC	Jia *et al*., 2020
18s	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG	Tengesdal *et al*., 2021

Table 1: Primer list for RT-qPCR (5' → 3' sequences).