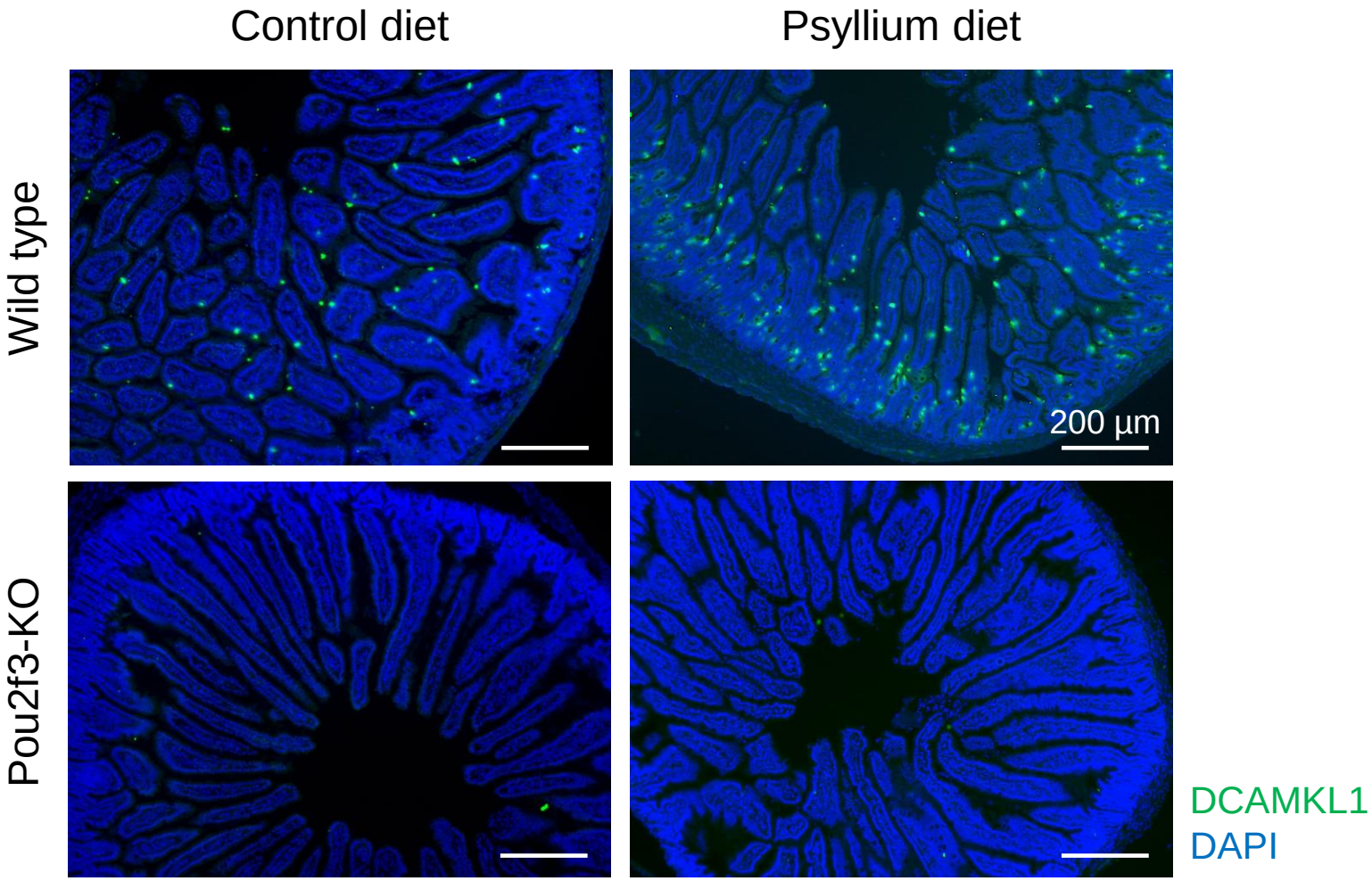
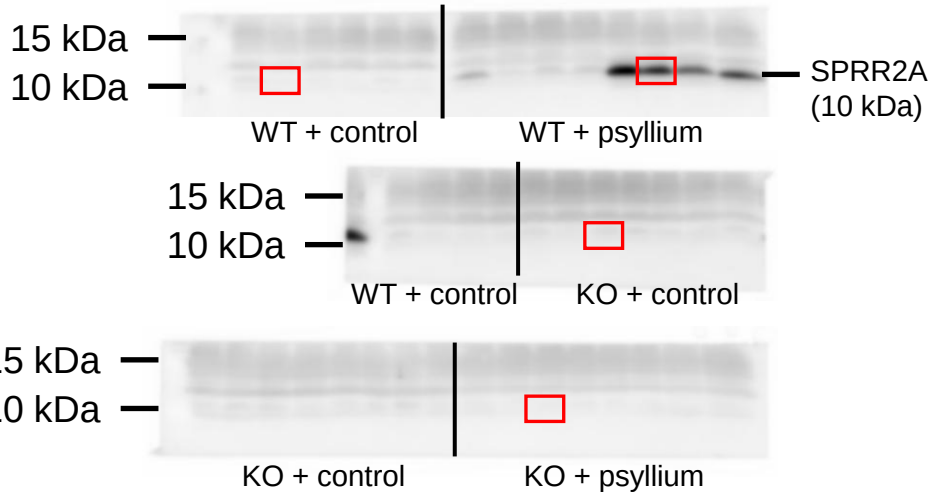
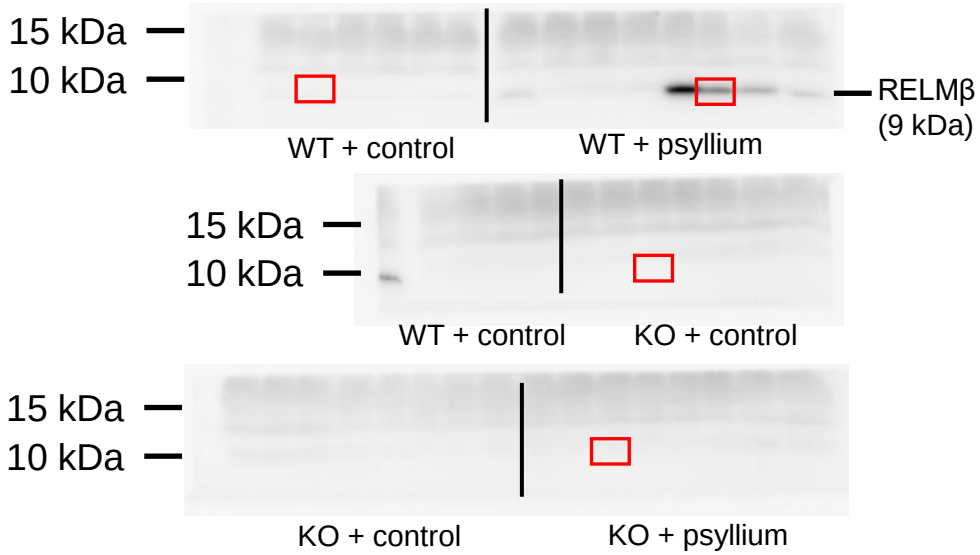


Figure S1. | Tuft cell expression (DCAMKL1) in psyllium supplementation.
Wild-type (WT) and tuft cell-deficient (Pou2f3-KO) mice were fed either a control diet or a 7.5% psyllium diet for 5 days. Representative immunofluorescence images of tuft cells marker (DCAMKL1, green), and counterstained with DAPI (blue) in jejunum.

Jejunum





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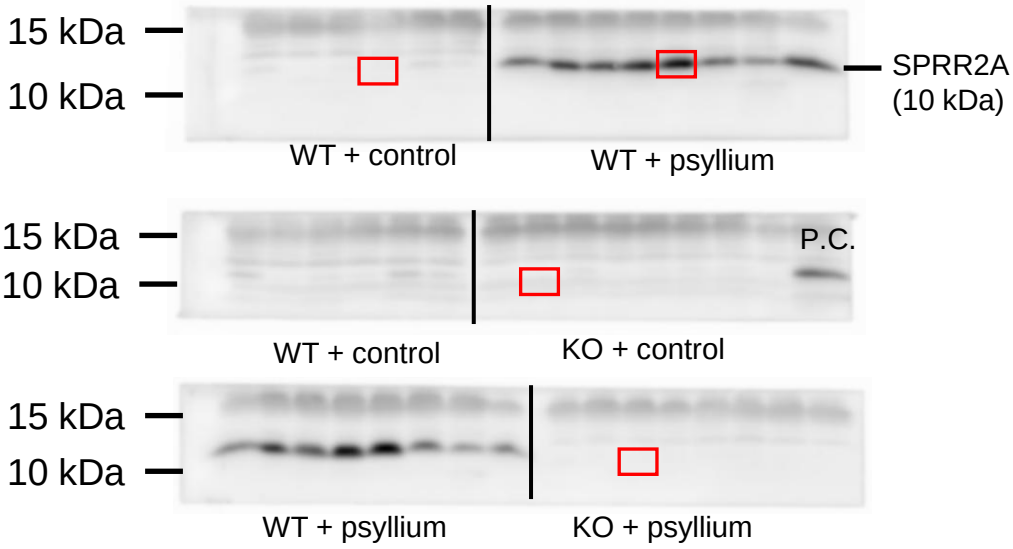

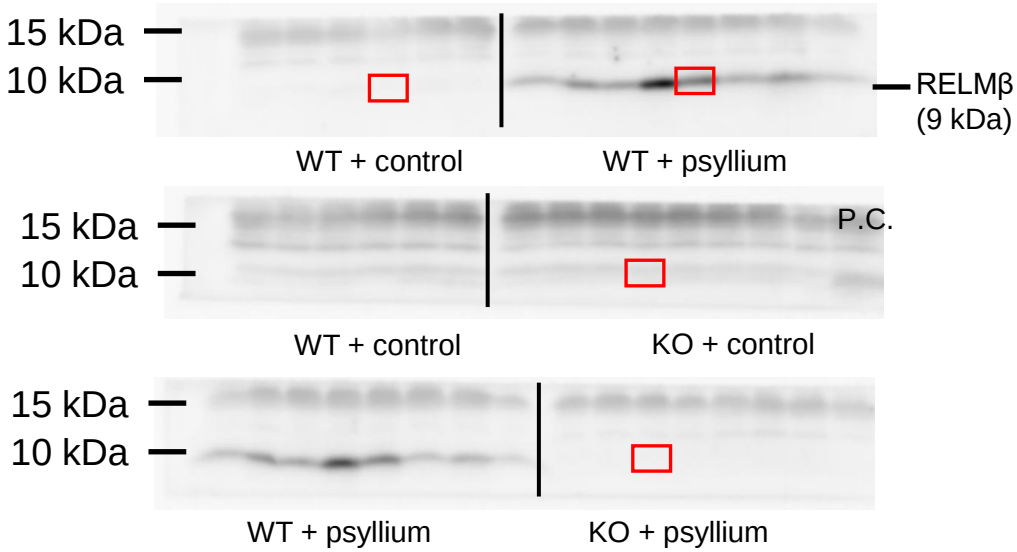

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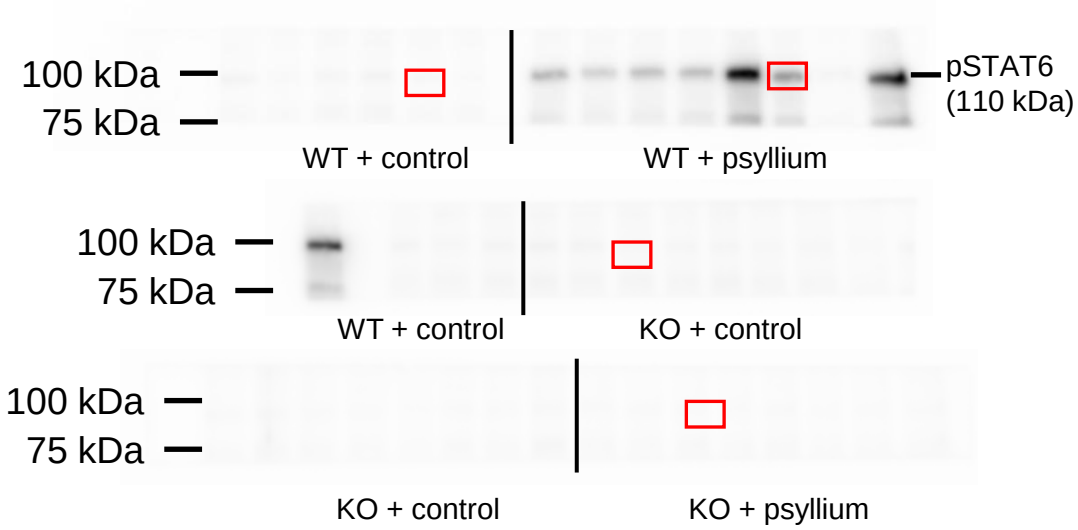
Jejunum



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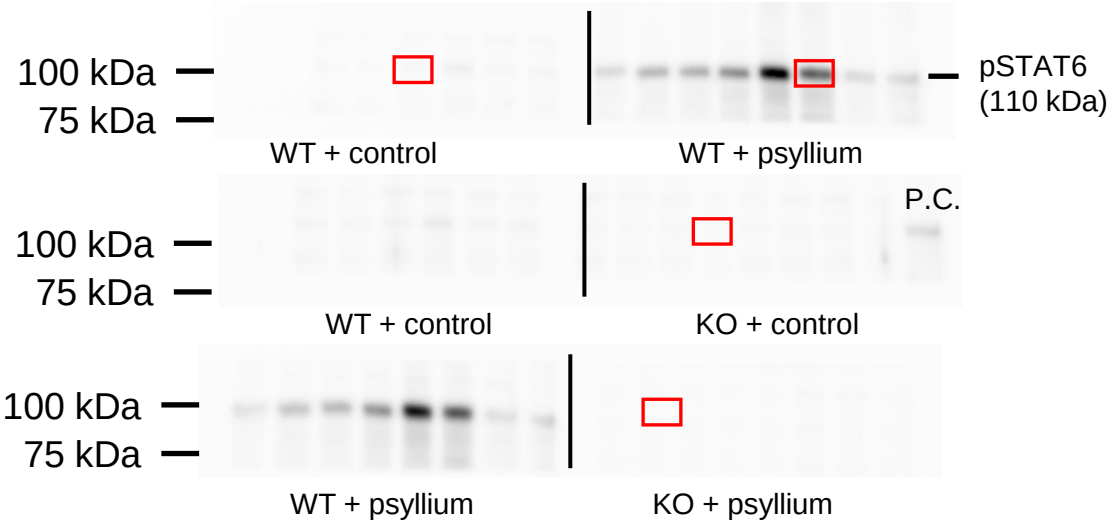

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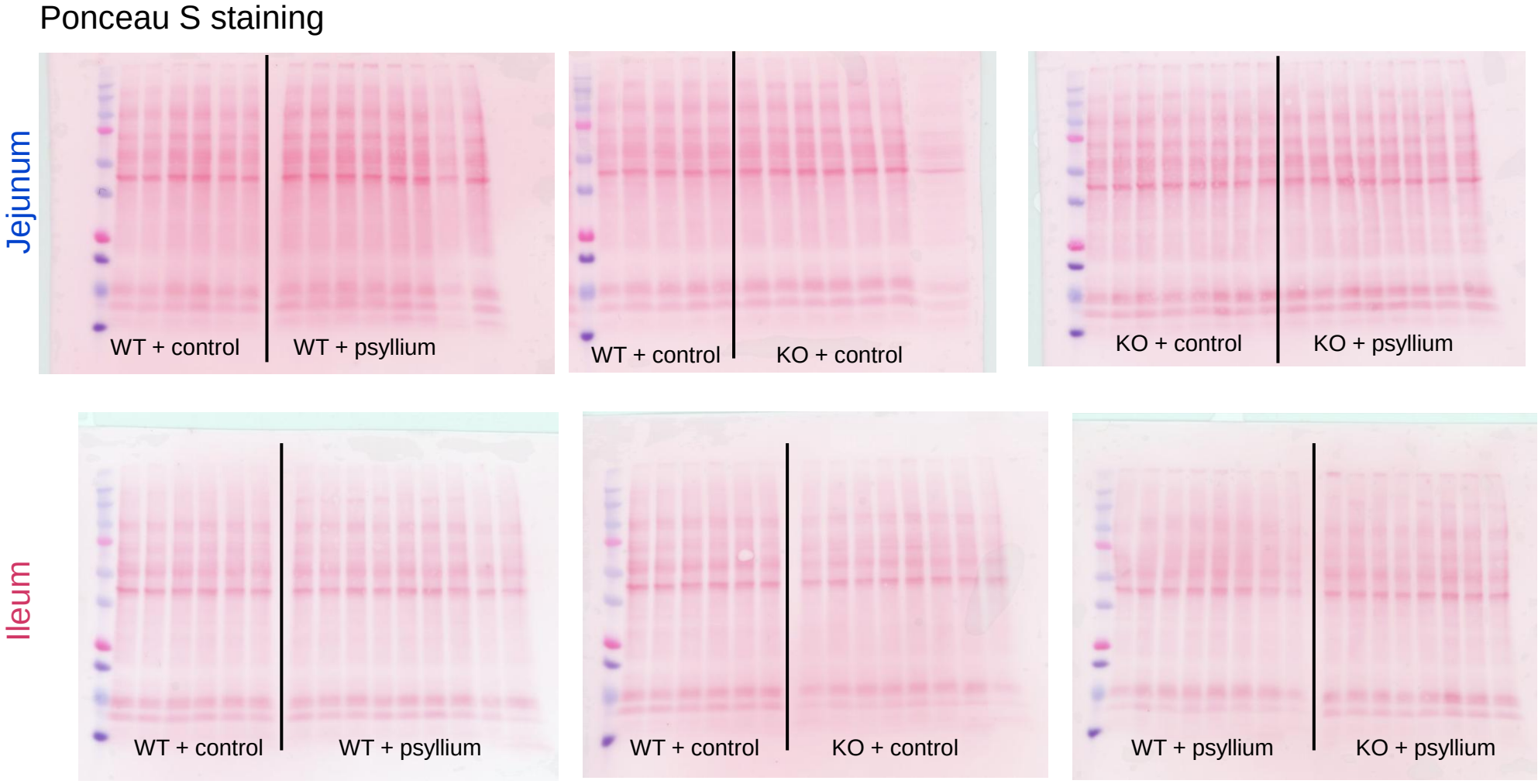


Figure S2. | Uncropped and unprocessed immunoblot images corresponding to the immunoblots shown in Fig. 1 and 2.
Wild-type (WT) and tuft cell-deficient (*Pou2f3*-KO) mice were fed either a control diet or a 7.5% psyllium diet for 5 days. Protein levels of SPRR2A, RELMβ, and STAT6 phosphorylation in the jejunum and ileum were assessed by immunoblotting (n = 6–8 per group). Immunoblot analyses for each target were performed using multiple gels run in parallel under identical experimental conditions. After transfer to PVDF membranes, total protein loading was visualized by Ponceau S staining and used for normalization. Membranes were cut horizontally according to molecular weight markers and probed separately with primary antibodies. Red boxes indicate the regions used for quantification and presentation in the main figures.

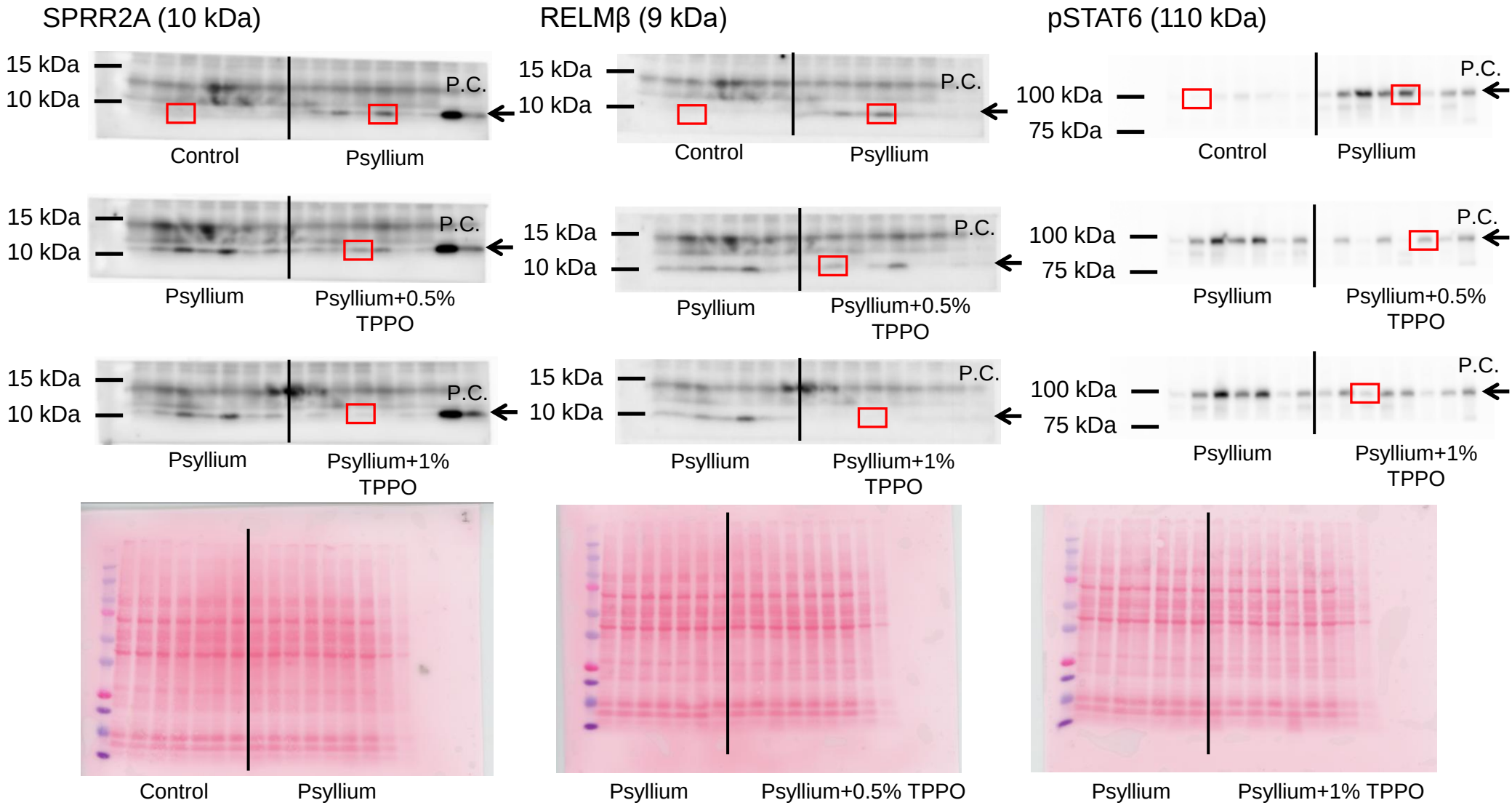


Figure S3. | Uncropped and unprocessed immunoblot images corresponding to the immunoblots shown in Fig. 3.
Mice were fed either a control diet or a 7.5% psyllium diet for 5 days. The TRPM5 inhibitor triphenylphosphine oxide (TPPO) was administered in the diet (0.5 or 1.0%, w/w), starting 1 day prior to psyllium feeding. Protein levels of SPRR2A, RELMβ, and STAT6 phosphorylation in the jejunum were assessed by immunoblotting (n = 7 per group). Immunoblot analyses for each target were performed using multiple gels run in parallel under identical experimental conditions. After transfer to PVDF membranes, total protein loading was visualized by Ponceau S staining and used for normalization. Membranes were cut horizontally according to molecular weight markers and probed separately with primary antibodies. Red boxes indicate the regions used for quantification and presentation in the main figures.

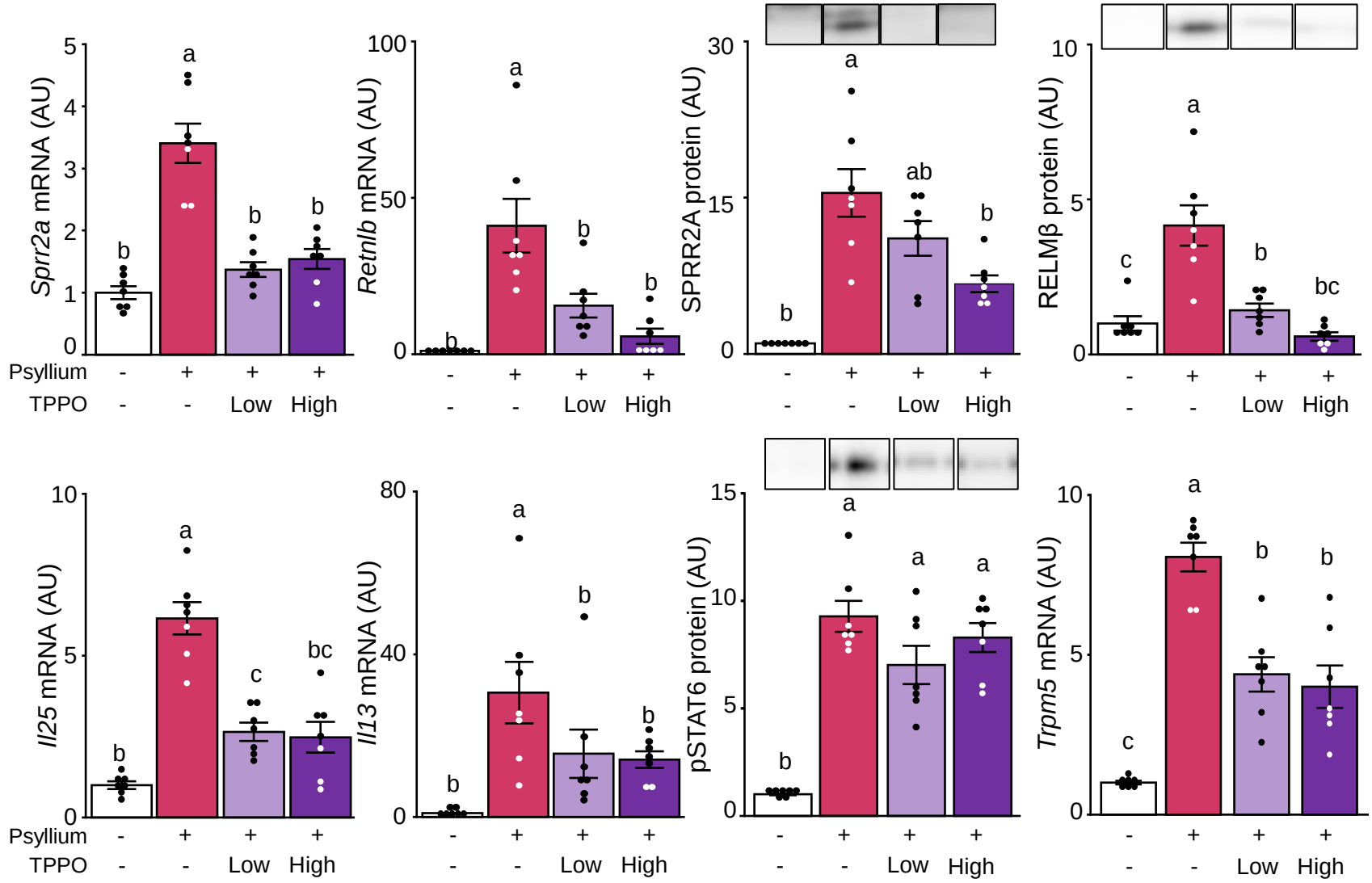


Figure S4. | Inhibition of TRPM5 signaling suppresses psyllium-induced ILC2 activation.
Mice were fed either a control diet or a 7.5% psyllium diet for 5 days. The TRPM5 inhibitor triphenylphosphine oxide (TPPO) was administered in the diet (0.5 or 1.0%, w/w), starting 1 day prior to psyllium feeding. Antimicrobial protein (SPRR2A, RELMβ), *Il25*, *Il13*, levels of STAT6 phosphorylation, and *Trpm5* expression was quantified by qRT-PCR or immunoblotting in mouse ileum (n=7 per group). Results are presented as mean ± s.e.m. Statistical significance was assessed by the Tukey–Kramer post-hoc test or the Steel–Dwass test. Groups not sharing a common letter are significantly different ($p < 0.05$).

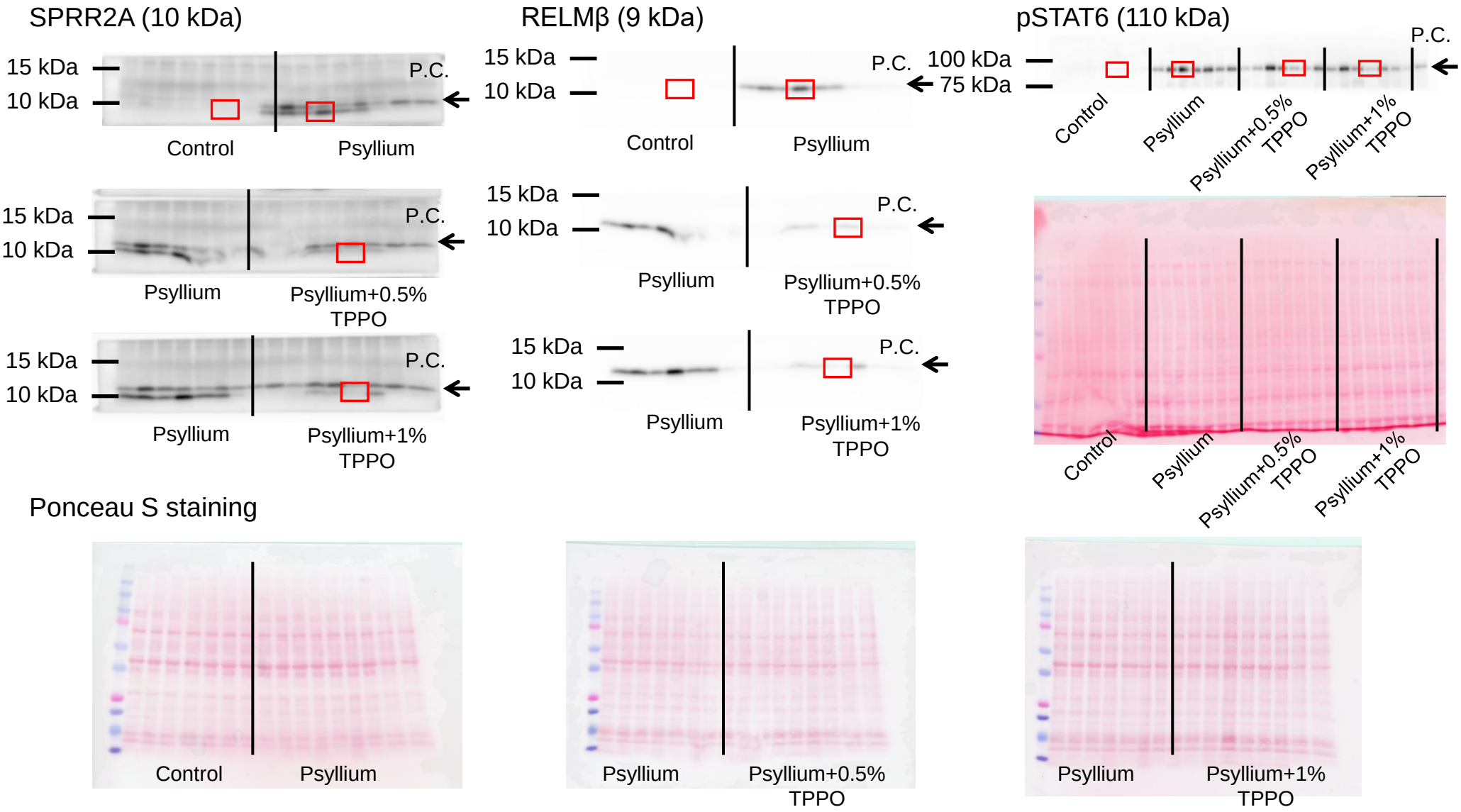


Figure S5. | Uncropped and unprocessed immunoblot images corresponding to the immunoblots shown in Fig. S4.
Mice were fed either a control diet or a 7.5% psyllium diet for 5 days. The TRPM5 inhibitor triphenylphosphine oxide (TPPO) was administered in the diet (0.5 or 1.0%, w/w), starting 1 day prior to psyllium feeding. Protein levels of SPRR2A, RELMβ, and STAT6 phosphorylation in the ileum were assessed by immunoblotting (n = 7 per group). Immunoblot analyses for each target were performed using multiple gels run in parallel under identical experimental conditions. After transfer to PVDF membranes, total protein loading was visualized by Ponceau S staining and used for normalization. Membranes were cut horizontally according to molecular weight markers and probed separately with primary antibodies.

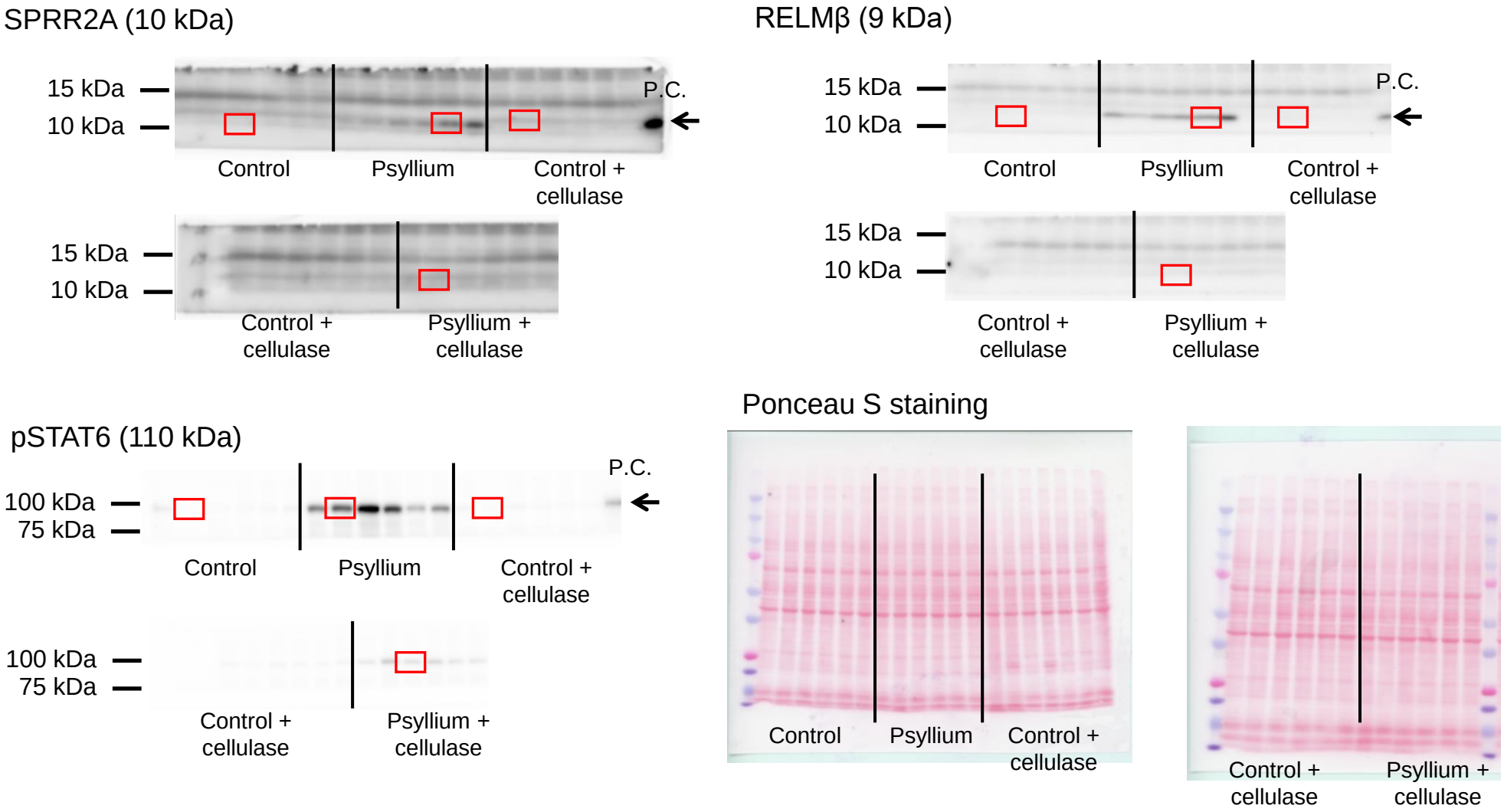


Figure S6. | Uncropped and unprocessed immunoblot images corresponding to the immunoblots shown in Fig. 4.
Mice were fed a 7.5% psyllium diet supplemented with and without cellulase preparation with xylanase activity for 5 days. Protein levels of SPRR2A, RELMβ, and STAT6 phosphorylation in the jejunum were assessed by immunoblotting (n = 6 per group). Immunoblot analyses for each target were performed using multiple gels run in parallel under identical experimental conditions. After transfer to PVDF membranes, total protein loading was visualized by Ponceau S staining and used for normalization. Membranes were cut horizontally according to molecular weight markers and probed separately with primary antibodies. Red boxes indicate the regions used for quantification and presentation in the main figures.

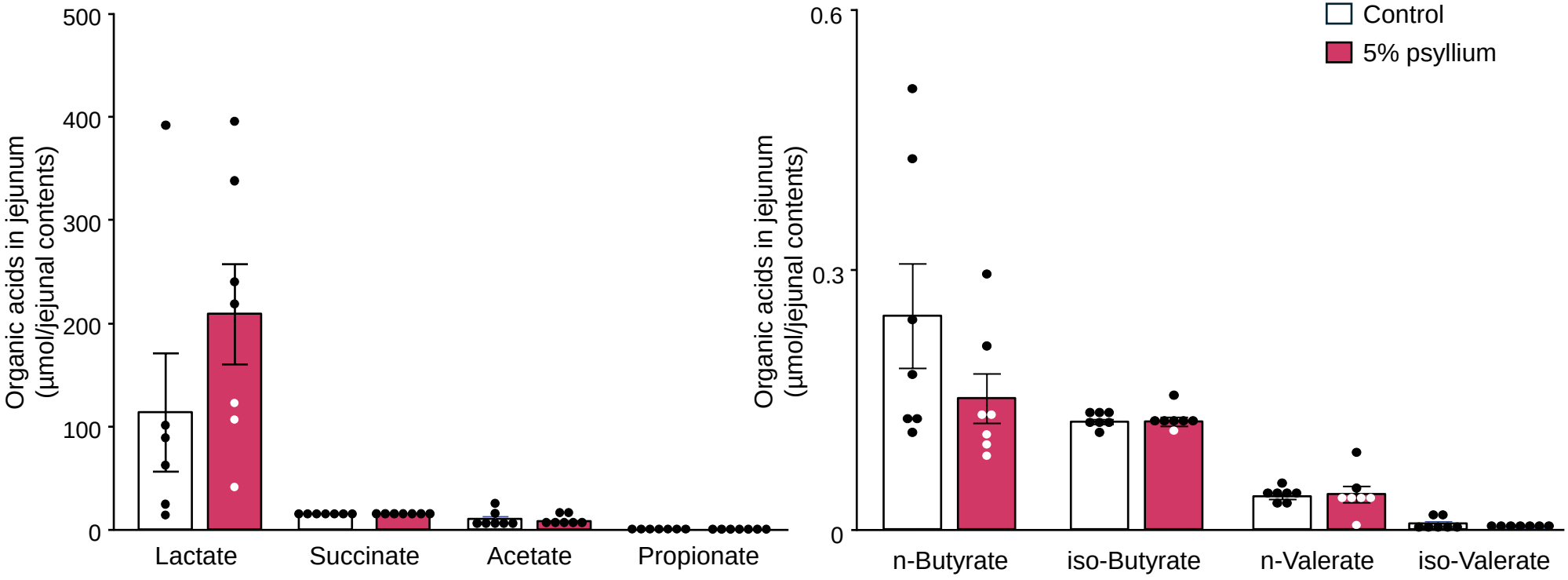


Figure S7. | Organic acid pools in mouse jejunal contents.
Mice were fed either a control diet or a 5% psyllium diet for 5 days. Lactate, succinate, acetate, propionate, n-butyrate, iso-butyrate, n-valerate, and iso-valerate were quantified by UPLC method (n=7 per group). Results are presented as mean ± s.e.m. Statistical significance was assessed by the Tukey–Kramer post-hoc test.

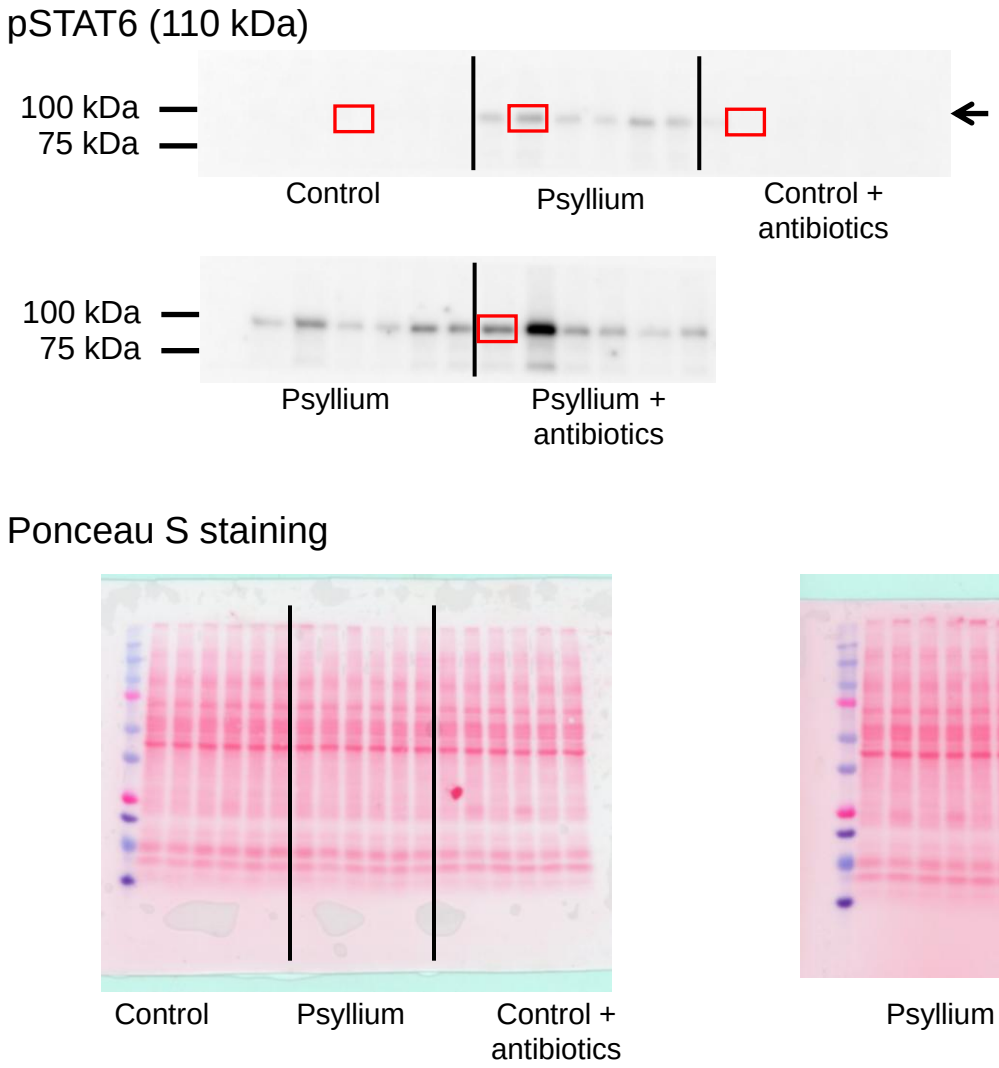
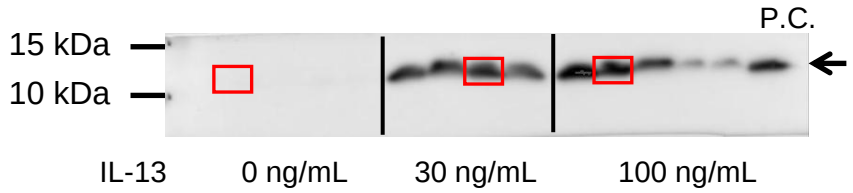
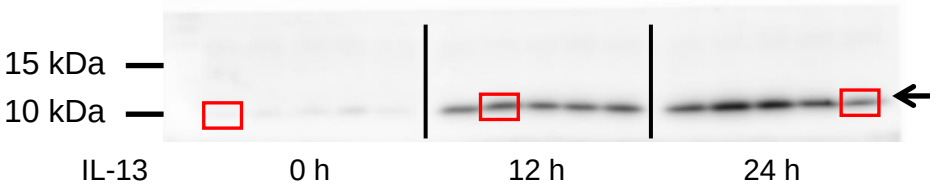
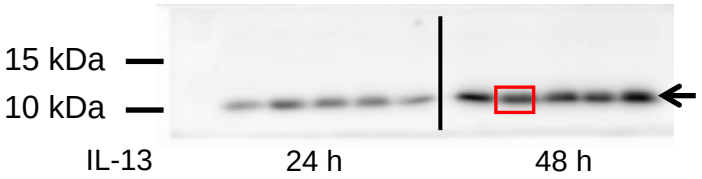


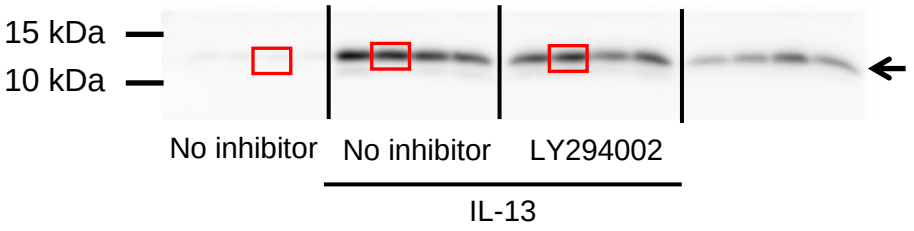
Figure S8. | Uncropped and unprocessed immunoblot images corresponding to the immunoblots shown in Fig. 5.
Mice were fed either a control diet or a 5% psyllium diet for 5 days. Antibiotics were administered via drinking water, starting 14 days before psyllium feeding. Protein levels of STAT6 phosphorylation in the jejunum were assessed by immunoblotting (n = 7 per group). Immunoblot analyses for each target were performed using multiple gels run in parallel under identical experimental conditions. After transfer to PVDF membranes, total protein loading was visualized by Ponceau S staining and used for normalization. Membranes were cut horizontally according to molecular weight markers and probed separately with primary antibodies. Red boxes indicate the regions used for quantification and presentation in the main figures.

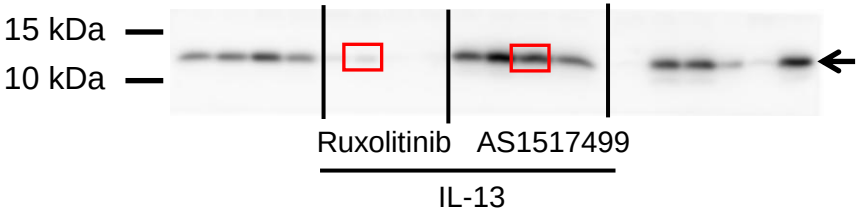
SPRR2A (10 kDa)



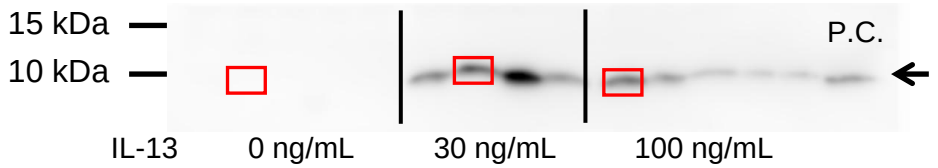


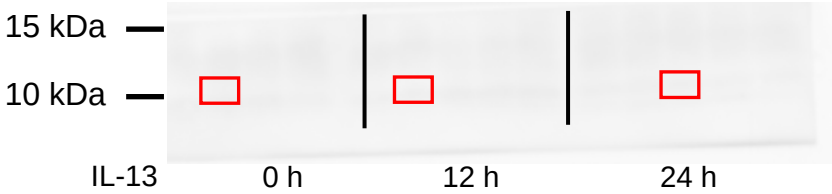


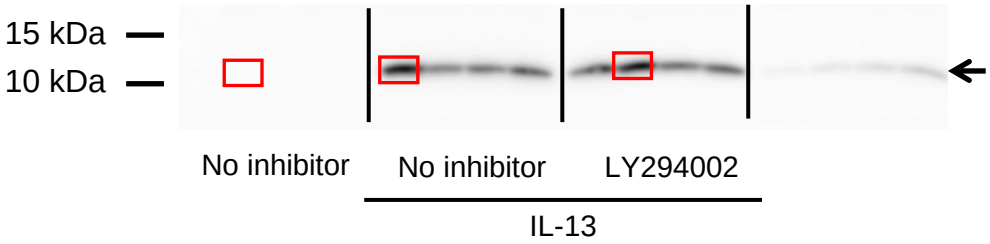


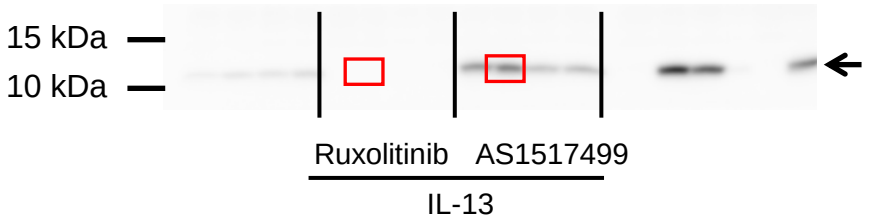


RELMβ (9 kDa)









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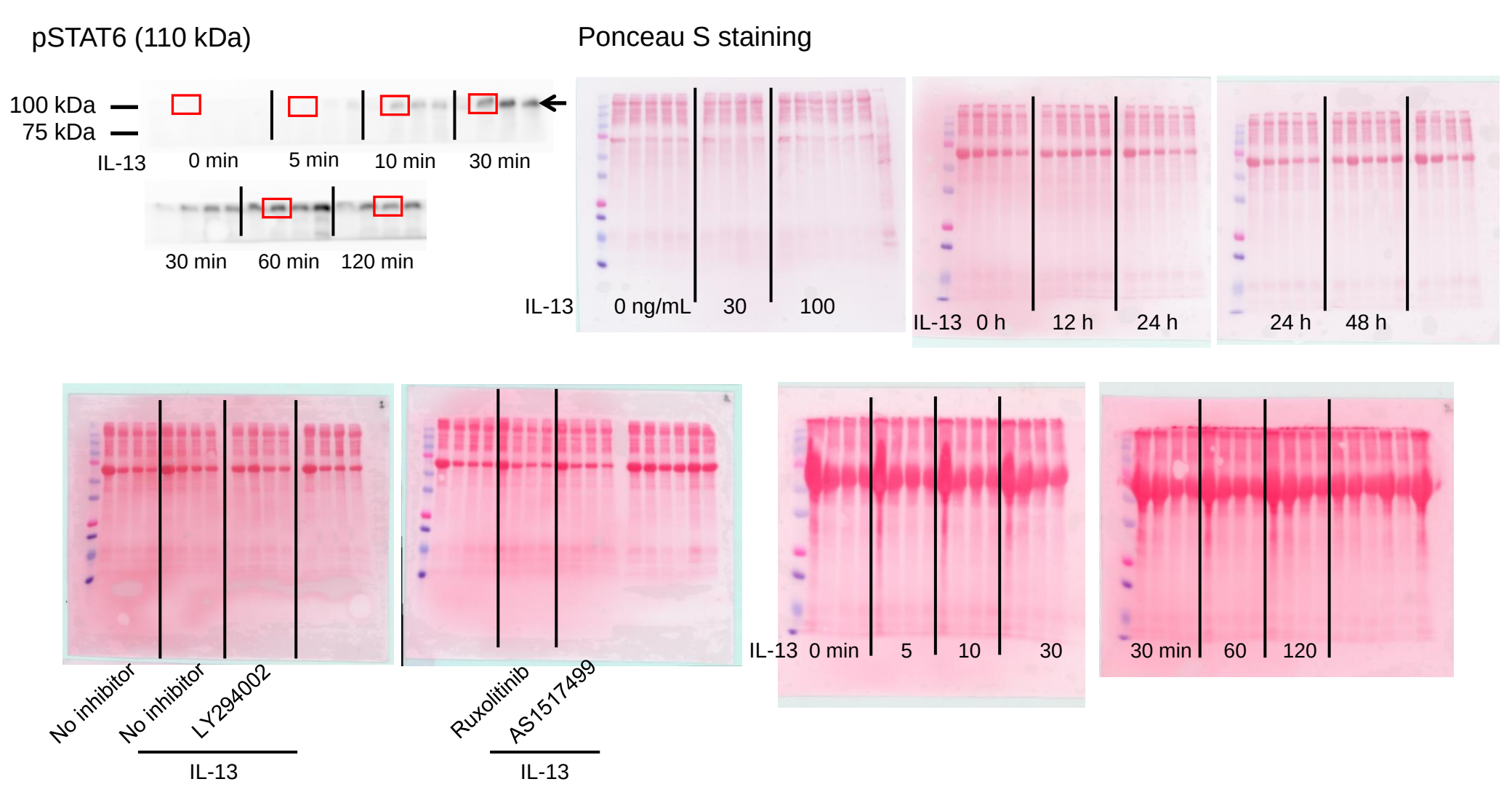


Figure S9. | Uncropped and unprocessed immunoblot images corresponding to the immunoblots shown in Fig. 6.
Organoids were established from mouse jejunum and stimulated with IL-13 (0, 30, or 100 ng/mL, n = 4-5 per group). Time-course analysis of AMP expression following the IL-13 stimulation for the indicated times (0–48 h, n = 5 per group). Organoids were treated with IL-13 (30 ng/mL) in the presence or absence of inhibitors targeting JAK1/2 [Ruxolitinib (10 µM)], STAT6 [AS1517499 (10 µM)], or PI3K [LY294002 (20 µM)]. Protein levels of SPRR2A, RELMβ, and STAT6 phosphorylation were assessed by immunoblotting (n = 4 per group). Immunoblot analyses for each target were performed using multiple gels run in parallel under identical experimental conditions. After transfer to PVDF membranes, total protein loading was visualized by Ponceau S staining and used for normalization. Membranes were cut horizontally according to molecular weight markers and probed separately with primary antibodies. Red boxes indicate the regions used for quantification and presentation in the main figures.

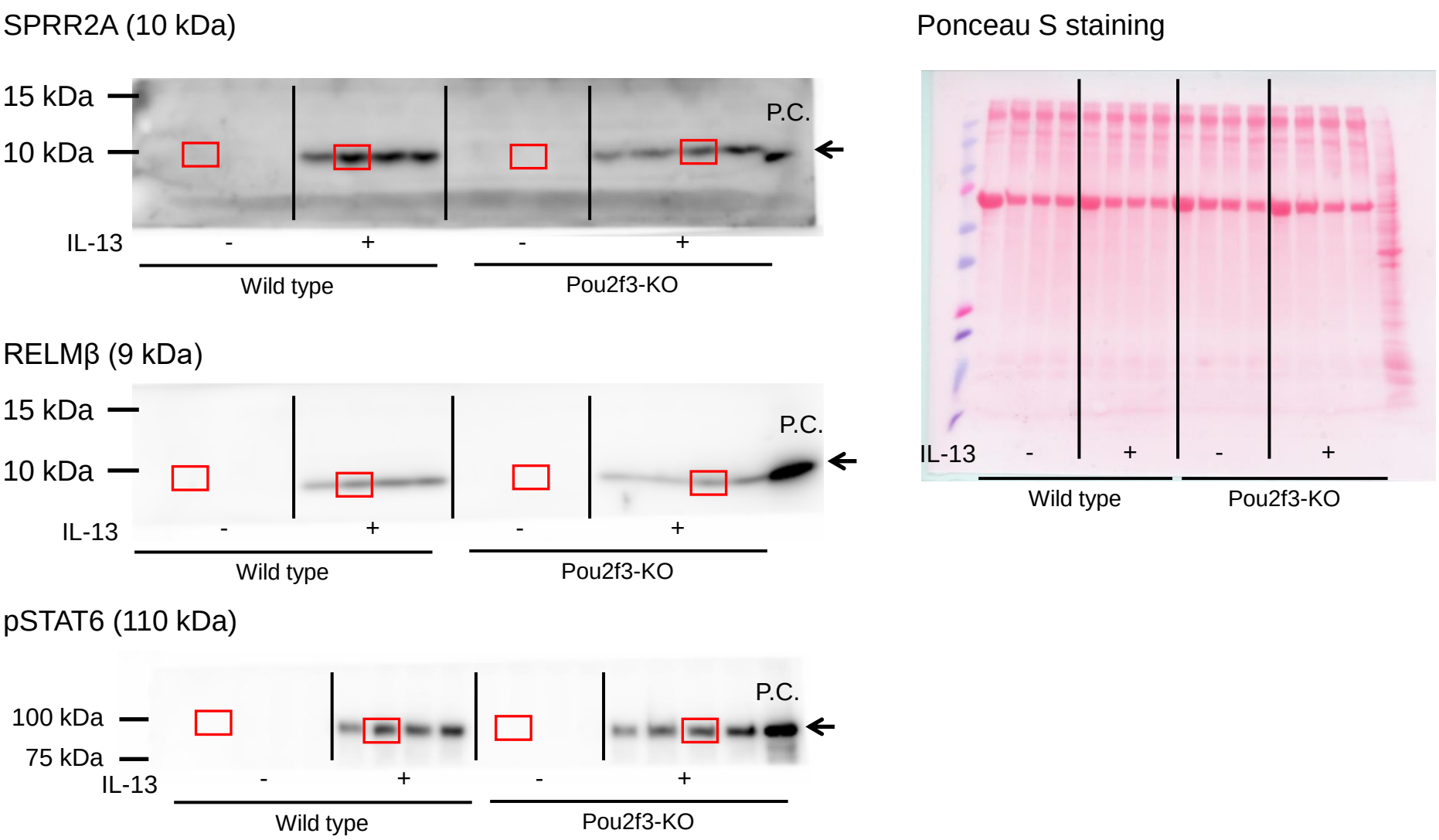


Figure S10. | Uncropped and unprocessed immunoblot images corresponding to the immunoblots shown in Fig. 7.
Organoids were established from the jejunum of WT and *Pou2f3*-KO mice and stimulated with or without IL-13 (30 ng/mL). Protein levels of SPRR2A, RELMβ, and STAT6 phosphorylation in the jejunum were assessed by immunoblotting (n = 7 per group). Immunoblot analyses for each target were performed using multiple gels run in parallel under identical experimental conditions. After transfer to PVDF membranes, total protein loading was visualized by Ponceau S staining and used for normalization. Membranes were cut horizontally according to molecular weight markers and probed separately with primary antibodies. Red boxes indicate the regions used for quantification and presentation in the main figures.

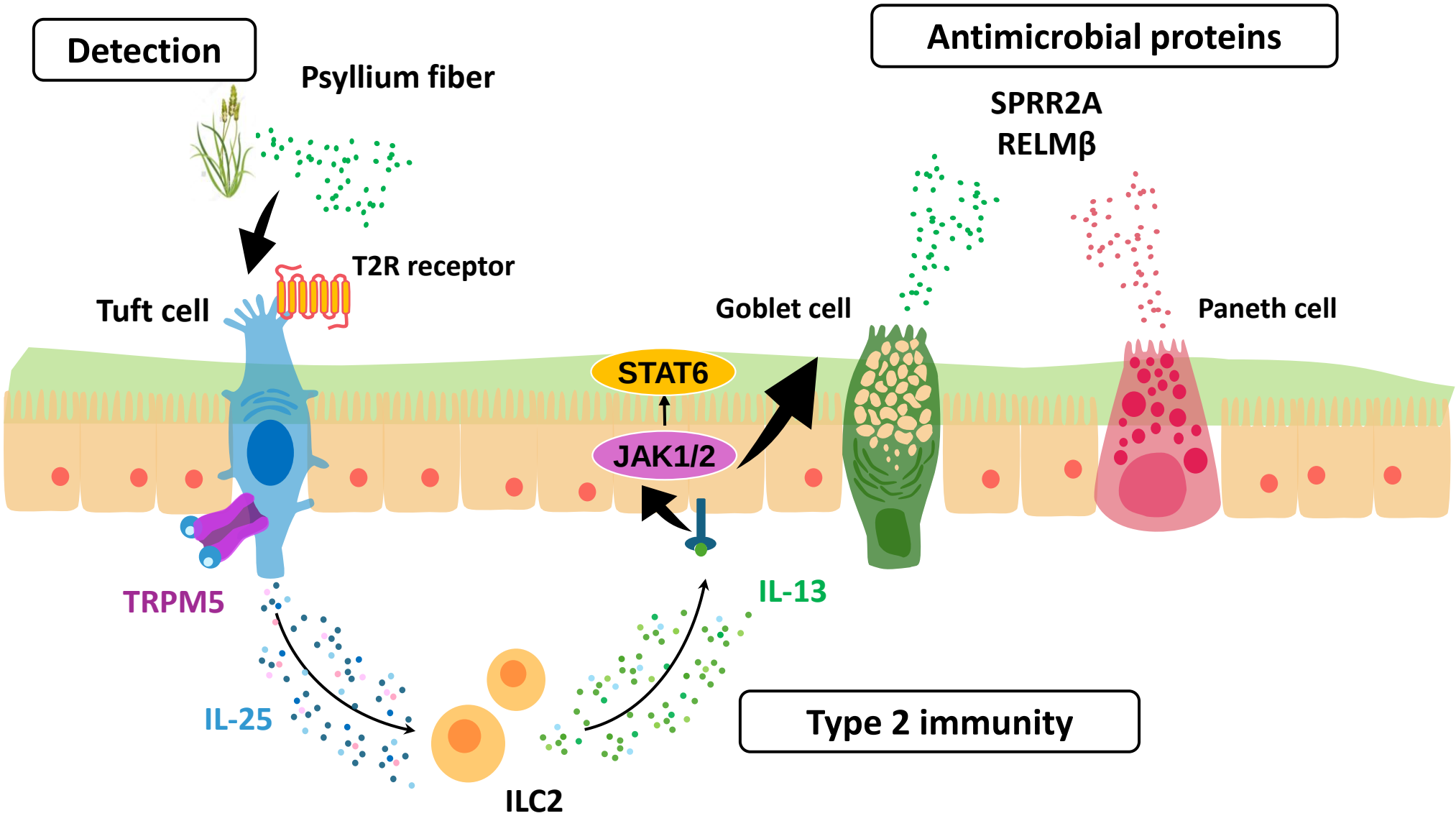
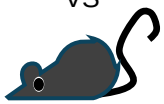


Figure S11. | Supplemental psyllium fiber upregulates antimicrobial protein (SPRR2A, RELMβ) production via tuft cell-ILC2-IL-13 circuit. Dietary psyllium fiber is sensed by intestinal tuft cells, triggering the release of IL-25 to activate ILC2s. This tuft cell–ILC2 axis drives the production of IL-13, which emerges as the principal effector promoting antimicrobial protein (SPRR2A, RELMβ) expression in intestinal epithelial cells via the JAK1/2–STAT6 pathway.

Experimant 1

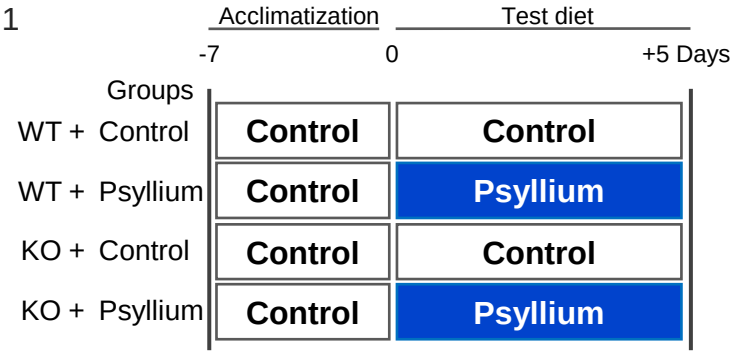
Wild type

VS



Pou2f3-KO

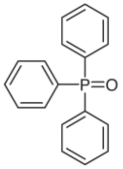
C57BL/6J, ♀
9 weeks, n=6 or 8



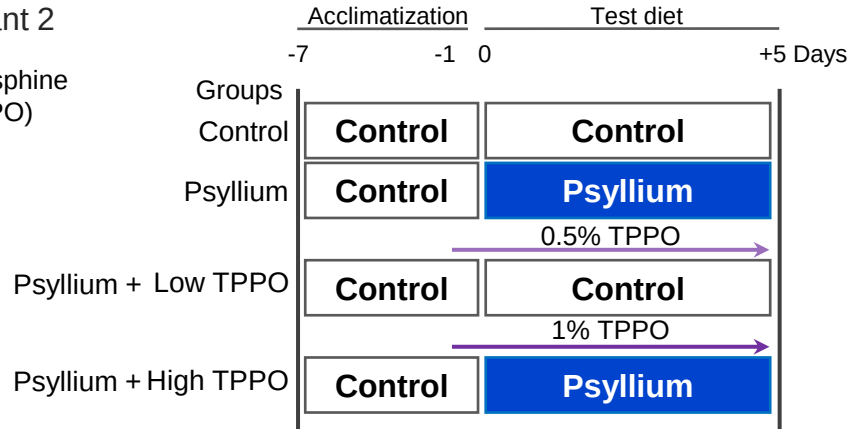
- qRT-PCR
- Immunoblot
- Immunofluorescence

Experimant 2

Triphenylphosphine oxide (TPPO)



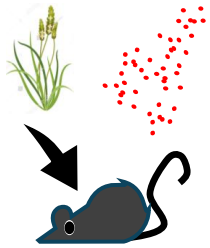
C57BL/6J, ♀
8 weeks, n=7



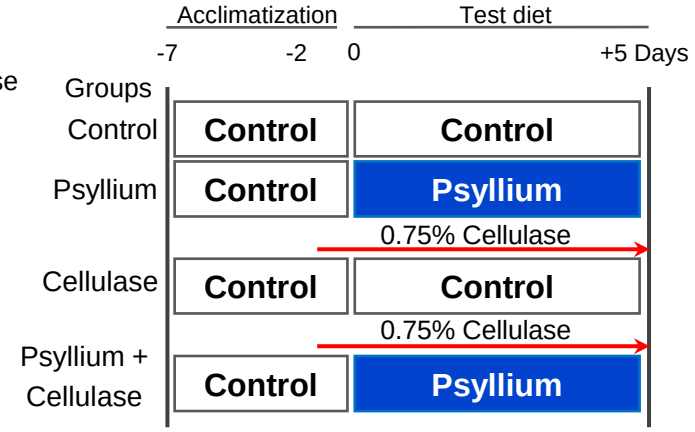
- qRT-PCR
- Immunoblot
- Immunofluorescence

Experimant 3

Psyllium Cellulase



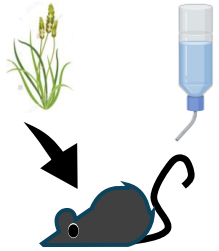
C57BL/6J, ♀
8 weeks, n=7



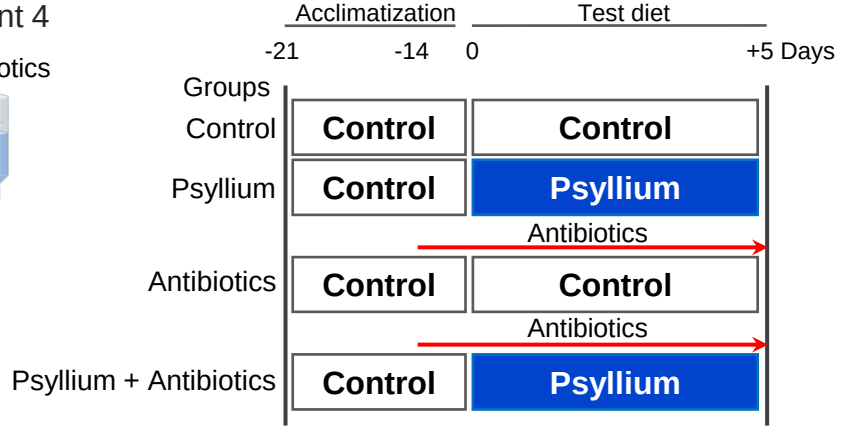
- qRT-PCR
- Immunoblot
- Immunofluorescence

Experimant 4

Psyllium Antibiotics



C57BL/6J, ♀
8 weeks, n=6



- qRT-PCR
- Immunoblot
- Immunofluorescence

Supplementary Figure continues on the next page.

Figure S12. | Experimental design and analytical workflow for animal experiments.
Schematic overview of the experimental schedules and analytical procedures for Experiments 1–4.

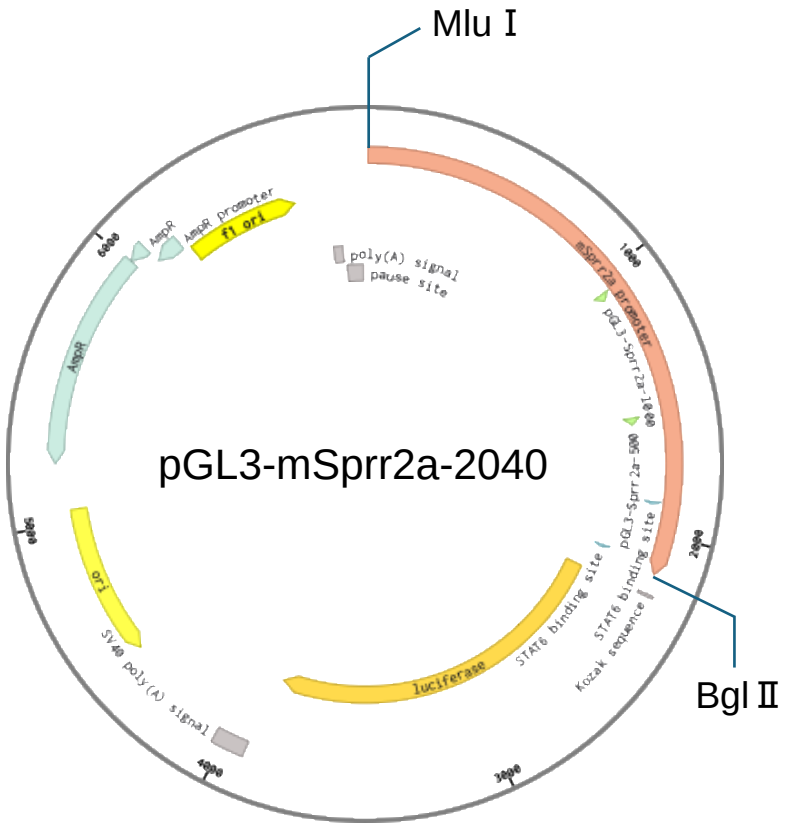
Experiment 1: To assess whether dietary psyllium fiber induces antimicrobial protein (AMP) production in the absence of tuft cells, wild-type (WT) and tuft cell–deficient Pou2f3 knockout (KO) mice were assigned to four groups: WT + control, WT + psyllium, KO + control, and KO + psyllium (n = 6 or 8 per group). Mice were fed control or 7.5% psyllium-containing diets for 5 days. Jejunal and ileal segments were collected for quantitative reverse transcription–polymerase chain reaction (qRT-PCR), immunoblotting, and immunofluorescence analyses.

Experiment 2: To examine the involvement of TRPM5-mediated chemosensory signaling, mice were treated with the TRPM5 inhibitor triphenylphosphine oxide (TPPO). Animals were allocated to four groups: control, psyllium, psyllium + 0.5% TPPO, and psyllium + 1.0% TPPO (n = 7 per group). The control group received the control diet for 5 days, whereas the other groups were fed a 7.5% psyllium diet. TPPO was incorporated into the diets at 0.5% or 1.0% (w/w), starting 1 day prior to psyllium feeding (total treatment period of 6 days). Jejunal and ileal tissues were subjected to qRT-PCR, immunoblotting, and immunofluorescence analyses.

Experiment 3: To determine whether the structural integrity of psyllium fiber is required for AMP induction, mice were assigned to four groups: control, psyllium, control + cellulase, and psyllium + cellulase (n = 6 per group). Control and control + cellulase groups were fed the control diet for 5 days, whereas the psyllium and psyllium + cellulase groups received a 7.5% psyllium diet. In the cellulase-treated groups, a cellulase preparation with xylanase activity was added to the diets at 0.75% (w/w). Jejunal and ileal segments were collected for qRT-PCR, immunoblotting, and immunofluorescence analyses.

Experiment 4: To evaluate the contribution of the gut microbiota to psyllium-mediated AMP induction, mice were randomly assigned to four groups: control, psyllium, control + antibiotics, and psyllium + antibiotics (n = 6 per group). Control and control + antibiotics groups were fed the control diet for 5 days, whereas psyllium and psyllium + antibiotics groups received a 5% psyllium diet. A broad-spectrum antibiotic cocktail was administered via drinking water starting 14 days before dietary intervention and continued throughout psyllium feeding (total of 19 days). Jejunal and ileal tissues were harvested for qRT-PCR, immunoblotting, and immunofluorescence analyses.

a



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 -841  GATGTTAGCA CACAGGTATT ATTTCTCTGT GTTTATGGAA AAAAATCTGT AGTAAATAGA
 -781  AGACCCTGAC TCCATGGCTT ACTGAGGGCG CACTTGGTAT CTTTTGCTTC TCTTCTTTCT
 -721  AAGAAACTTA TAAATATATG AGGAAAATAC CCCTCCACAT CTGAAAAAAA AAAGTGTAAA
 -661  TGGCTTTACA CTCTAGCAAC GAACTAAGGA TGAACTAAAG AGGGACAAAT AACCTTGAAT
 -601  TTAAGACAAA TAGAGGGTCT CATGAAAAAA GGCTTATGGT CCCCAGTCAA GAAAGAGATG
 -541  TATCAAGCGG TTGGAAAAGC TCCAAGTACC ACAGTTACTG GAAGCAAGAG GAAAGAAAAG
 -481  GACTCTGAGT CATAAGACTC AACCTAGTAA TGATAGCCAA GGGTGGGATA TTTTCTATTT
 -421  TGTAGAGTCC CTGTCAGGCC AGTTATGGAT GAATGTGCAC TTAAGTTATC TCCTTCTAGC
 -361  CTAGCAAGGC TGGCTGTGAC CATCTGCTGC CCCTGGAGCA GAGCCACCTG CTGTGTTGCT
 -301  CTTTTTGATG TGCCACTACC TGCCTGAGGC CCAACCTGCC TGAGGCTGAA TGAGTTCTCT
 -241  TCAAGGAATC TTCTGGAGTT AACACTGAAC TGCAATCTTC ACGAGTTCCC ACTAAAAGGC
 -181  TGAACTATCT ACTTCTAAGG AACTTGCTTG GCAAGGGAAG GGCTTCCCCT TTTCTTTACA
 -121  AAGCATGTTT GCTGACATTA AATTTGAACC TTGAACAGGA AGCTTGTCTT GGTTTCTTTC
  -61  TTATCACAGG CAGCCCAGCC CTTATTCTCT TTCAGGTTTC CAAAATGCCT TTCCAGAATA
   +1  GAACCCAGCT CTGTGATCTG CTGGCCAGAC ACAACACCTG TACCAGCCCA TTACAGGGAA
  +61  ATCCACTCCC CATGGGGTGA GGCAGGCAAT CCTAT
```

-1000 bp
-500 bp
STAT6 binding site
TSS
STAT6 binding site

c

WT ---TTCTAAGGAA---
-168bp Mut. ---CGTTAAGAGC---

WT ---TTACAGGGAA---
+51bp Mut. ---CGGCAGGAGC---

Figure S13. | Construction of the Sprr2a promoter–luciferase reporter plasmids.
(a) Schematic map of the mouse Sprr2a promoter–luciferase reporter construct generated in the pGL3 basic vector. The Sprr2a promoter region spanning −2040 to +95 bp relative to the major transcription start site was cloned into the pGL3 vector using the MluI and BglII restriction sites. The plasmid map was designed using Benchling software. (b) Nucleotide sequence of the Sprr2a promoter region. Putative STAT6-binding motifs are underlined. (c) For the -168 bp site, the sequence 5′-TTC-3′ and 5′-GAA-3′ were mutated to 5′-CGT-3′ and 5′-AGC-3′, respectively. For the +51 bp site, the sequence 5′-TTA-3′ and 5′-GAA-3′ were mutated to 5′-CGG-3′ and 5′-AGC-3′, respectively.