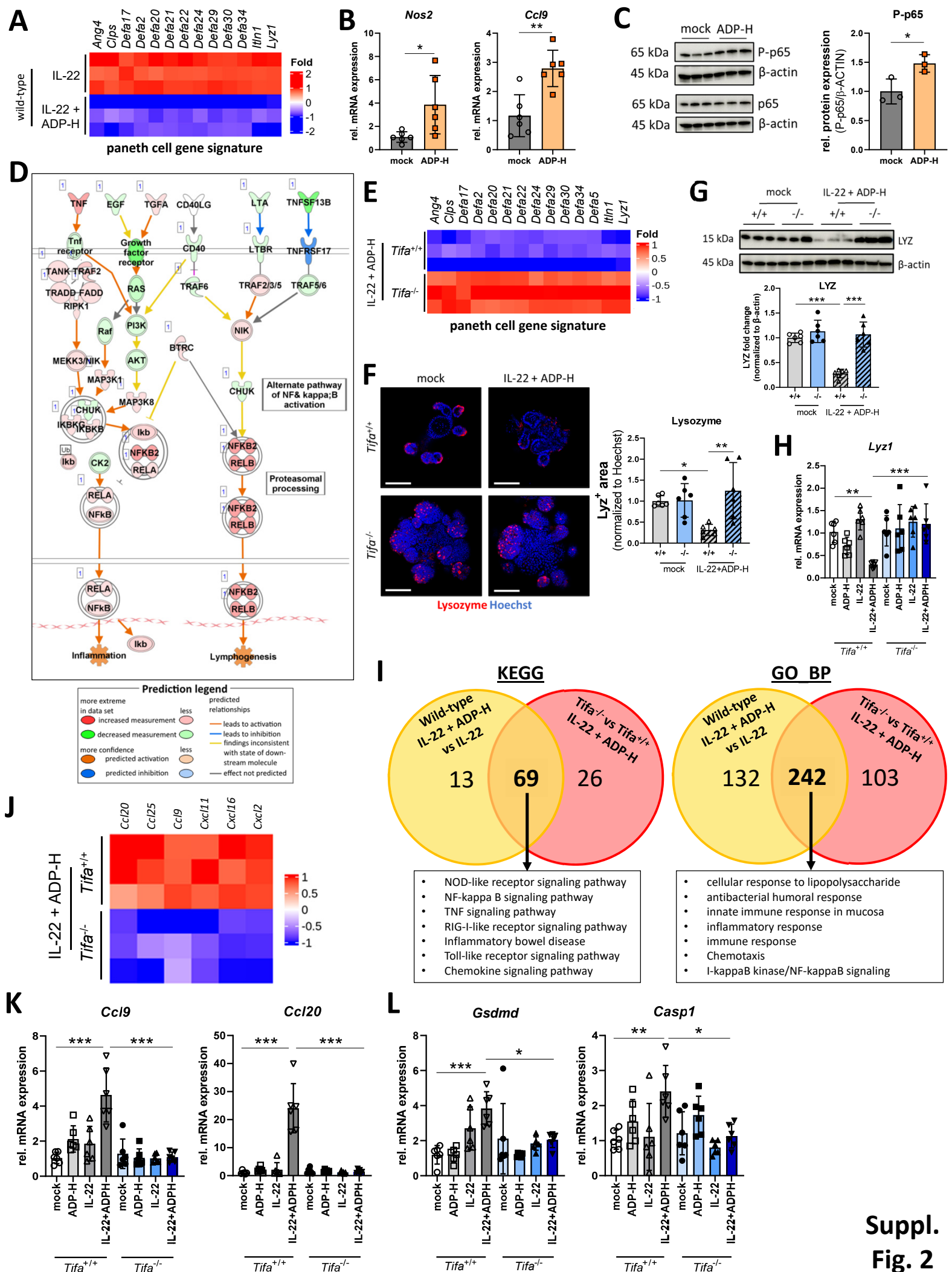


**Suppl. Fig. 1: TIFA as mediator of immune-epithelial communication.**

(A) qPCR analysis of *Tifa* mRNA levels in different murine inflammatory models (n = 5-8/group). (B) Representative FISH staining images of *Tifa* mRNA in the ileum of specific-pathogen-free (SPF) and germ-free (GF) mice (scale bar 100 μm) (n = 7-8/group). (C) *Tifa* counts in the colon of GF mice compared to SPF mice (n = 3-4/group). (D) *Tifa* expression in Si organoids stimulated with the indicated toll-like receptor ligands (n = 3/group). (E) Schematic of the IL-22 minicircle construct and experimental procedure. Overall, data are presented as mean ± SD. (F) Murine Si organoid monolayer cultures, all transfected with a TIFA-mScarlet2 reporter protein (red), stimulated with or without IL-22 for 8 hours (scale bar 100 μm) (3 independent experiments). \*\*\* indicates p < 0.001 by one-way ANOVA (Tukey's post hoc test: A) or Mann-Whitney test (C).

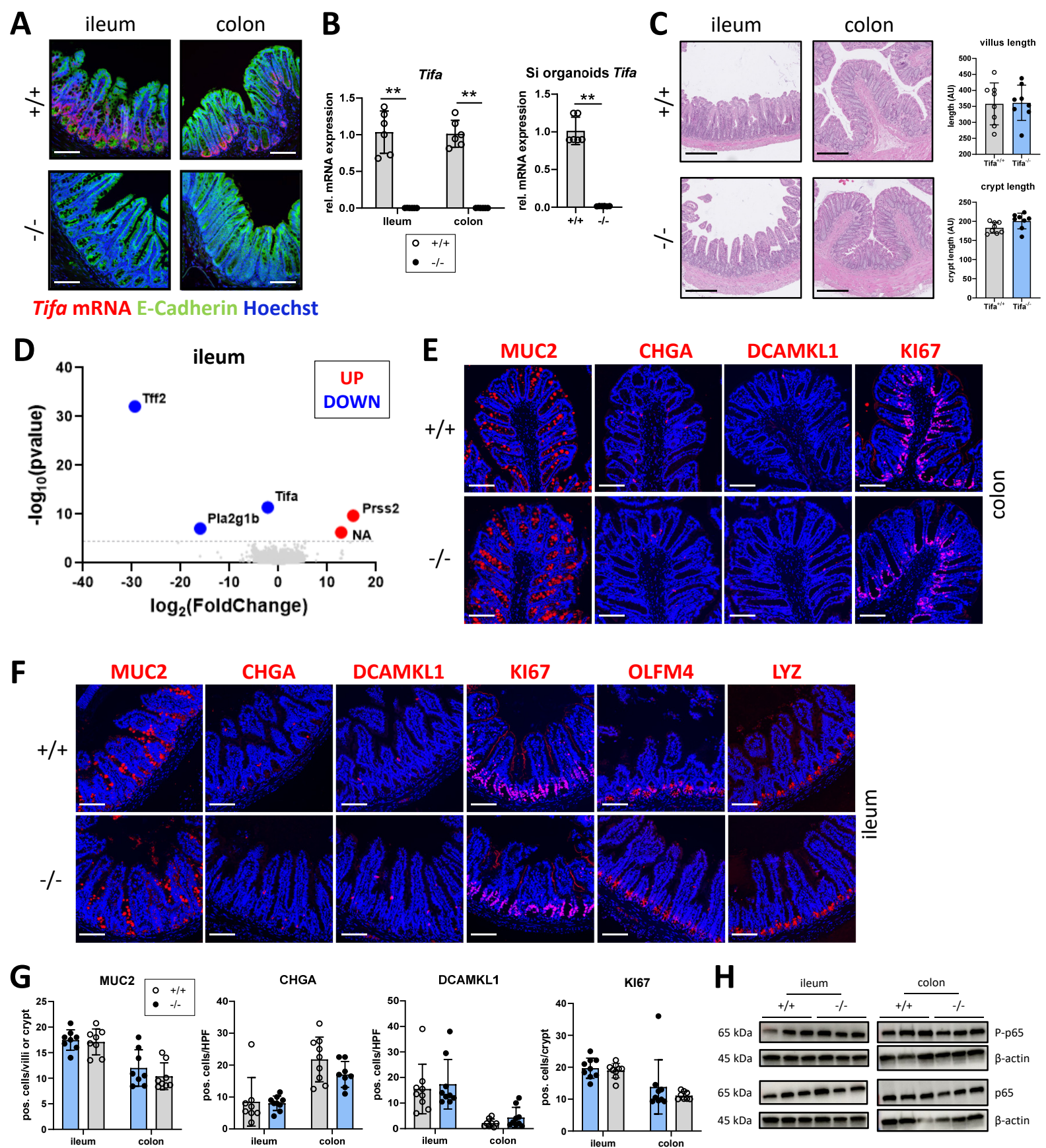


Suppl.  
Fig. 2

**Suppl. Fig. 2: ADP-heptose signaling in intestinal epithelial cells is strictly dependent on TIFA.**

**(A)** Heatmap showing a Paneth cell gene signature in wild-type Si organoids stimulated with IL-22 and with or without ADP-H. **(B)** qPCR analysis for *Nos2* and *Ccl9* mRNA expression levels in freshly isolated IECs from the small intestine of wild-type mice, embedded in Matrigel, cultured for 48 hours and then stimulated with 25 µg/ml ADP-H for 24 hours (n = 6/group). **(C)** Western blot analysis of P-p65 and p65 of freshly isolated IECs from the small intestine of wild-type mice, embedded in Matrigel, cultured for 48 hours and then stimulated with 25 µg/ml ADP-H for 24 hours. β-actin was used as loading control (n = 3/group). **(D)** Canonical pathway analysis generated by Ingenuity Pathway Analysis (IPA, Qiagen) of wild-type Si organoids treated with IL-22 and ADP-H showing upregulation (red) and predicted activation (orange) of molecules involved in the NF-κB signaling pathway. **(E)** Heatmap showing a Paneth cell gene signature in Si organoids from *Tifa*<sup>-/-</sup> versus *Tifa*<sup>+/+</sup> mice stimulated with IL-22 and ADP-H. **(F)** Representative immunofluorescence staining images for the Paneth cell marker lysozyme of Si organoids from *Tifa*<sup>+/+</sup> and *Tifa*<sup>-/-</sup> mice stimulated with or without ADP-H and IL-22 with analysis of the LYZ<sup>+</sup> area (normalized to Hoechst as counterstain) (scale bar 100µm) (n = 6/group). **(G)** Western blot analysis of lysozyme (LYZ) from Si organoids of *Tifa*<sup>+/+</sup> and *Tifa*<sup>-/-</sup> mice stimulated with or without IL-22 and ADP-H. β-actin was used as loading control (n = 6/group). **(H)** *Lyz1* gene expression from *Tifa*<sup>-/-</sup> and *Tifa*<sup>+/+</sup> Si organoids stimulated with IL-22 and ADP-H (n = 6/group). **(I)** Selected KEGG and gene ontology biological process (GO\_BP) analysis, showing overlapping pathways of wild-type Si organoids stimulated with IL-22 and ADP-H versus IL-22 alone compared to pathways affected in Si organoids from *Tifa*<sup>-/-</sup> and *Tifa*<sup>+/+</sup> mice stimulated with IL-22 and ADP-H. **(J)** Heatmap of transcripts encoding chemokines from the RNAseq approach. **(K)** *Ccl9* and *Ccl20* gene expression from *Tifa*<sup>-/-</sup> and *Tifa*<sup>+/+</sup> Si organoids stimulated with IL-22 and ADP-H (n = 6/group). **(L)** *Gsdmd* and *Casp1* gene expression from *Tifa*<sup>-/-</sup> and *Tifa*<sup>+/+</sup> Si organoids stimulated with IL-22 and ADP-H (n = 6/group). Overall, data are presented as mean ± SD. \*, \*\* and \*\*\* indicates p < 0.05, p < 0.01 and p < 0.001, respectively by Mann-Whitney test (B), Student's t-test (C) or one-way ANOVA (Tukey's post hoc test: F, G, H, K, L).





Suppl.  
Fig. 3

**Suppl. Fig.3: *Tifa*<sup>-/-</sup> mice have a normal intestinal phenotype.**

**(A)** Representative FISH staining images of *Tifa* mRNA expression (red) in the ileum and colon of *Tifa*<sup>+/+</sup> and *Tifa*<sup>-/-</sup> mice, counterstained with E-Cadherin (green) (scale bar 100  $\mu$ m) (3 independent experiments; n = 7/group). **(B)** *Tifa* gene expression in ileum, colon and Si organoids of *Tifa*<sup>+/+</sup> and *Tifa*<sup>-/-</sup> mice (n = 6/group). **(C)** Representative H&E images of ileal and colonic sections from *Tifa*<sup>+/+</sup> and *Tifa*<sup>-/-</sup> mice with assessment of villus length and crypt length (scale bar 250  $\mu$ m) (3 independent experiments; n = 8/group). **(D)** Volcano plot analysis of RNA sequencing data from ileal samples of *Tifa*<sup>-/-</sup> mice compared to littermate *Tifa*<sup>+/+</sup> mice. Upregulated genes are shown in red, downregulated genes in blue (n = 3/group). **(E, F)** Representative immunofluorescence images of colon (E) and ileum (F) from *Tifa*<sup>+/+</sup> and *Tifa*<sup>-/-</sup> mice for MUC2 (goblet cells), Chromogranin A (CHGA; enteroendocrine cells), DCAMKL1 (tuft cells), KI67 (proliferation), OLFM4 (stem cells), and lysozyme (LYZ; Paneth cells) (scale bar 100  $\mu$ m) (3 independent experiments; n = 8-9/group) **(G)** Assessment of positively stained cells in MUC2, CHGA, DCAMKL1 or KI67 immunofluorescence stainings counted per high power field (HPF) (n = 8-9/group). **(H)** Western blot analysis of phospho-p65 (P-p65) and p65 protein expression in the ileum and colon of unchallenged *Tifa*<sup>+/+</sup> and *Tifa*<sup>-/-</sup> mice (n = 5-6/group). Overall, data are presented as mean  $\pm$  SD. \*\* indicates p < 0.01, by one-way ANOVA (Sidak test: B (ileum, colon), G) or Mann-Whitney test (C: Si organoids).