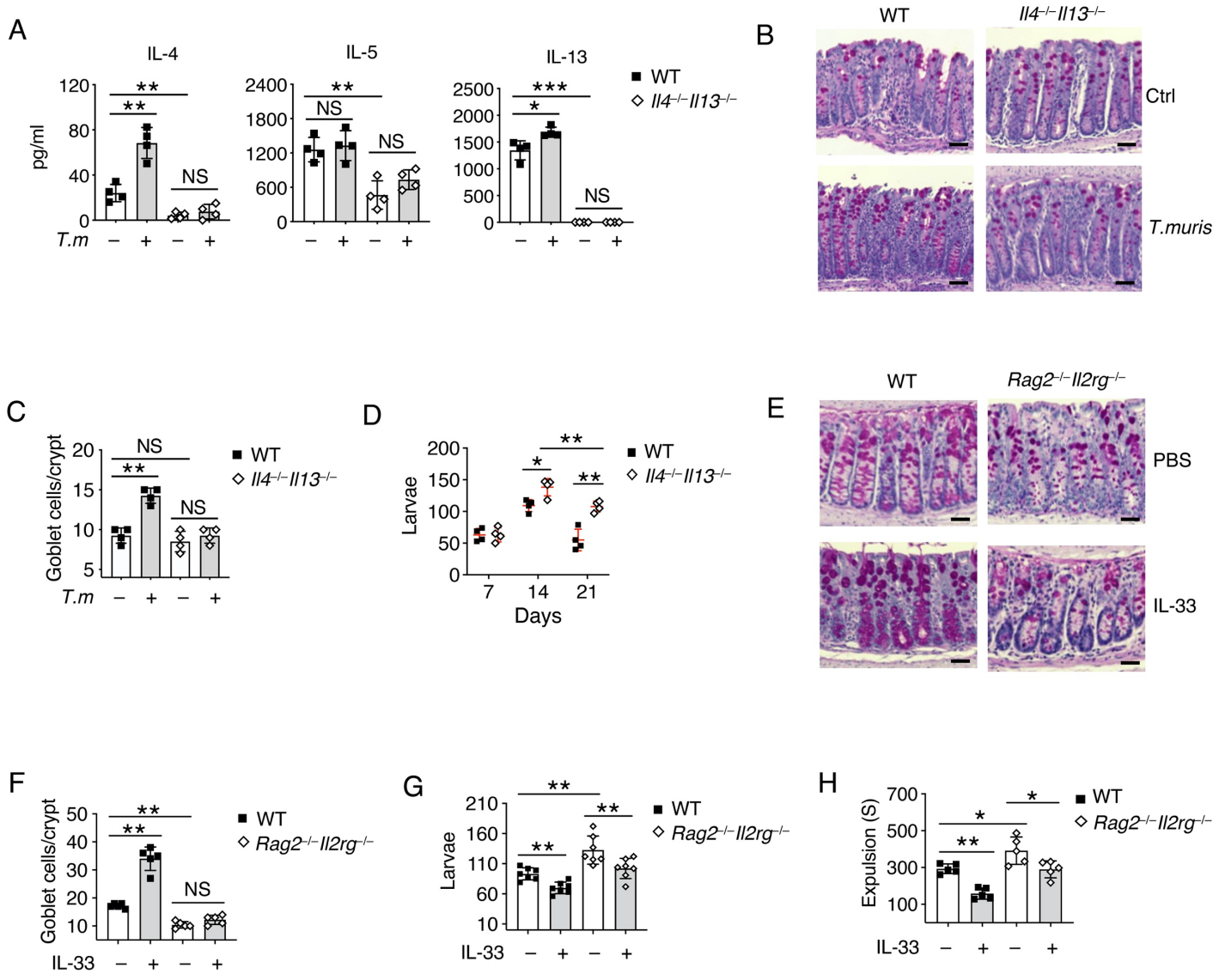

Figure S1. *Il4*$^{-/-}$*Il13*$^{-/-}$ mice exhibit reduced goblet cells during *T. Muris* infection, related to Figure 1.

(A-C) *T. muris* eggs were inoculated into WT and *Il4*$^{-/-}$*Il13*$^{-/-}$ mice by oral gavage. The mice were sacrificed on day 14 for further testing.

(A) IL-4, IL-5 and IL-13 protein levels from mLN with or without *T. Muris* infection by were assessed ELISA.

(B) PAS staining and of colon WT and (C) Quantification of goblet cells per crypt from colonic tissues with or without *T. Muris* infection. Scale bar, 50 μm.

(D) *T. muris* eggs were inoculated into WT and *Il4*$^{-/-}$*Il13*$^{-/-}$ mice by oral gavage. The mice were sacrificed on day 7, 14 and 21 to quantify of larvae in the colonic tissues.

(E-H) *T. muris* eggs were inoculated into WT and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice by oral gavage. The mice were treated with PBS or IL-33 daily from day 7 and sacrificed on day 14 for further test.

(E) PAS staining and (F) Quantification of goblet cells per crypt of colonic tissues. Scale bar, 50 μm.

(G) Quantification of larvae in the colons of WT and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice.

(H) *T. muris* infected WT and *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice were treated daily with PBS or IL-33 for 7 days. Colonic transit time was assessed by bead expulsion assay.

Data are representative of three independent experiments (A-H). NS, not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ (Student's *t*-test, error bars represent SD).

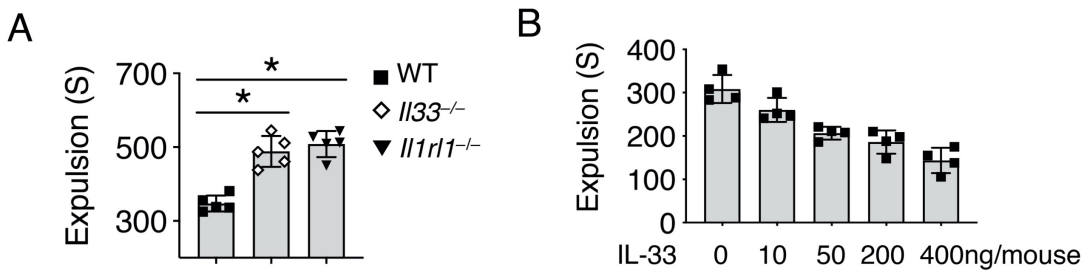

Figure S2. IL-33-ST2 signal regulates gut motility, related to Figure 2.

(A) WT and *Il1rl1*$^{-/-}$ mice were treated with antibiotics for 4 weeks. Colonic transit time was measured by bead expulsion assay.

(B) WT mice were treated with the indicated dosage of IL-33 for 10 minutes. Colonic transit time was measured by bead expulsion assay.

Data are representative of three independent experiments (B) or are pooled from two independent experiments (A). *$p < 0.05$ (Student's *t*-test, error bars represent SD).

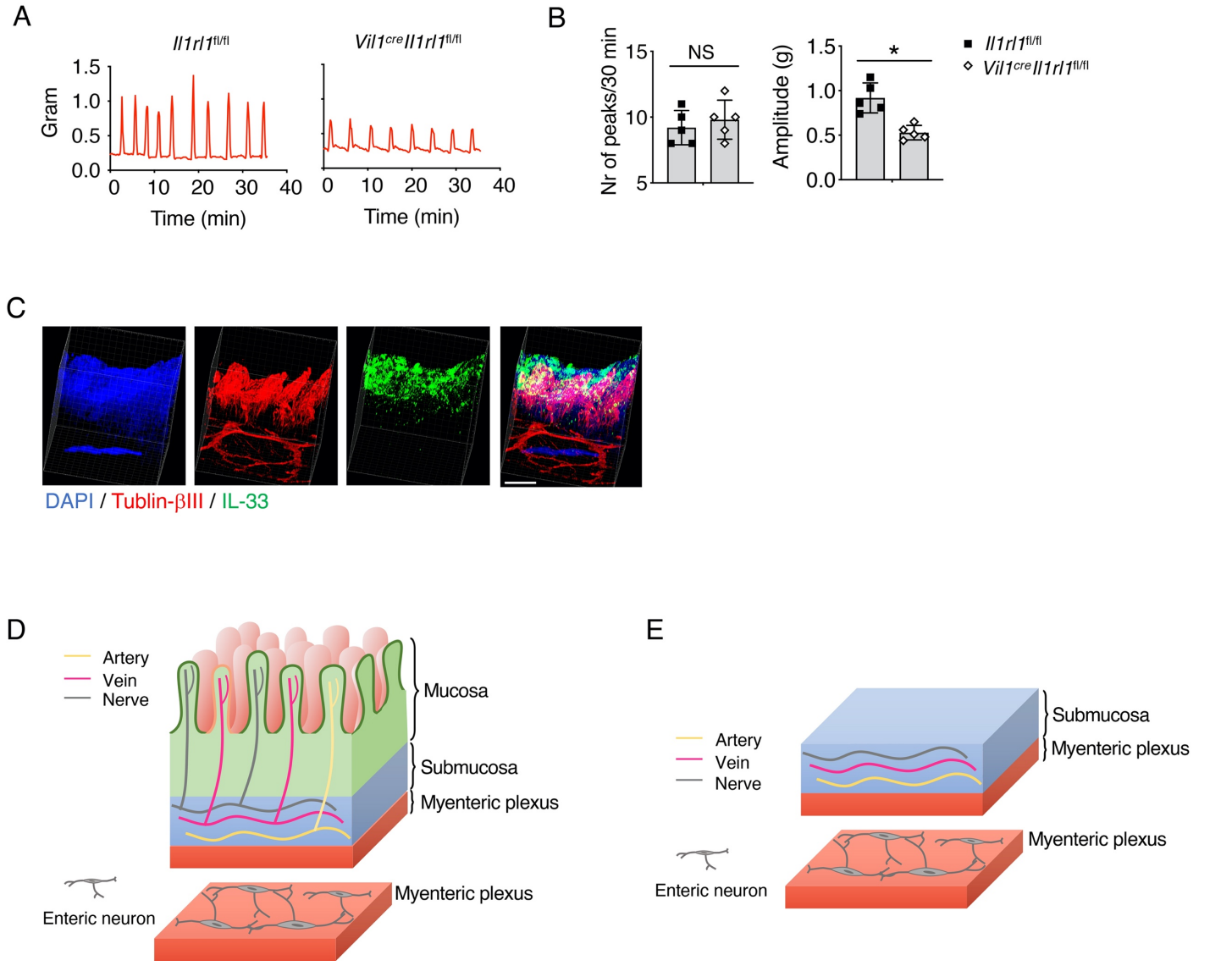

Figure S3 IEC-derived ST2 is required for gut motility, related to Figure 3.

(A) Representative trace and (B) Quantification of colon contraction in *Il1rl1*$^{fl/fl}$ and *Vil1*^{cre}*Il1rl1*$^{fl/fl}$ mice.

(C) iDISCO from the intestine isolated from *Il33*^{GFP} mouse.

(D) Illustration of anatomy of intact intestinal tissue.

(E) Illustration of anatomy of intestinal tissue without the epithelium.

Data are representative of three independent experiments (A, C) or are pooled from two independent experiments (B). NS, not significant; *$p < 0.05$ (Student's *t*-test, error bars represent SD).

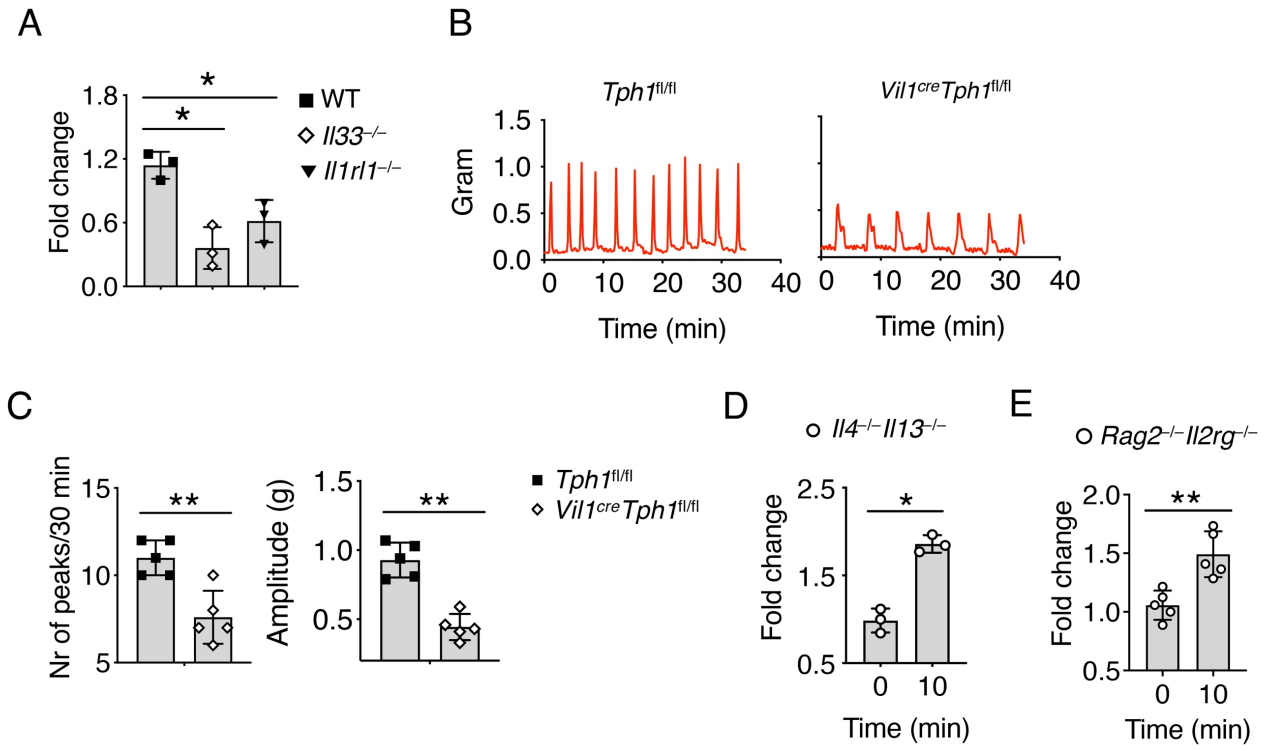

Figure S4. IL-33 induces 5-HT secretion for gut motility, related to Figure 4.

(A) Relative 5-HT levels in WT, *Il33*$^{-/-}$ and *Il1rl1*$^{-/-}$ mice serum were assessed by ELISA.

(B) Representative trace and (C) Quantification of colon contraction in *Tph1*$^{fl/fl}$ and *Vil1*^{cre}*Tph1*$^{fl/fl}$ mice.

(D) *Il4*$^{-/-}$*Il13*$^{-/-}$ (E) *Rag2*$^{-/-}$*Il2rg*$^{-/-}$ mice were i.p. injected with IL-33 and waited for 10 minutes. Mice serum was collected, and relative 5-HT levels were assessed by ELISA.

Data are representative of three independent experiments (A, B, D, E) or are pooled from two independent experiments (C). *$p < 0.05$; **$p < 0.01$ (Student's *t*-test, error bars represent SD).

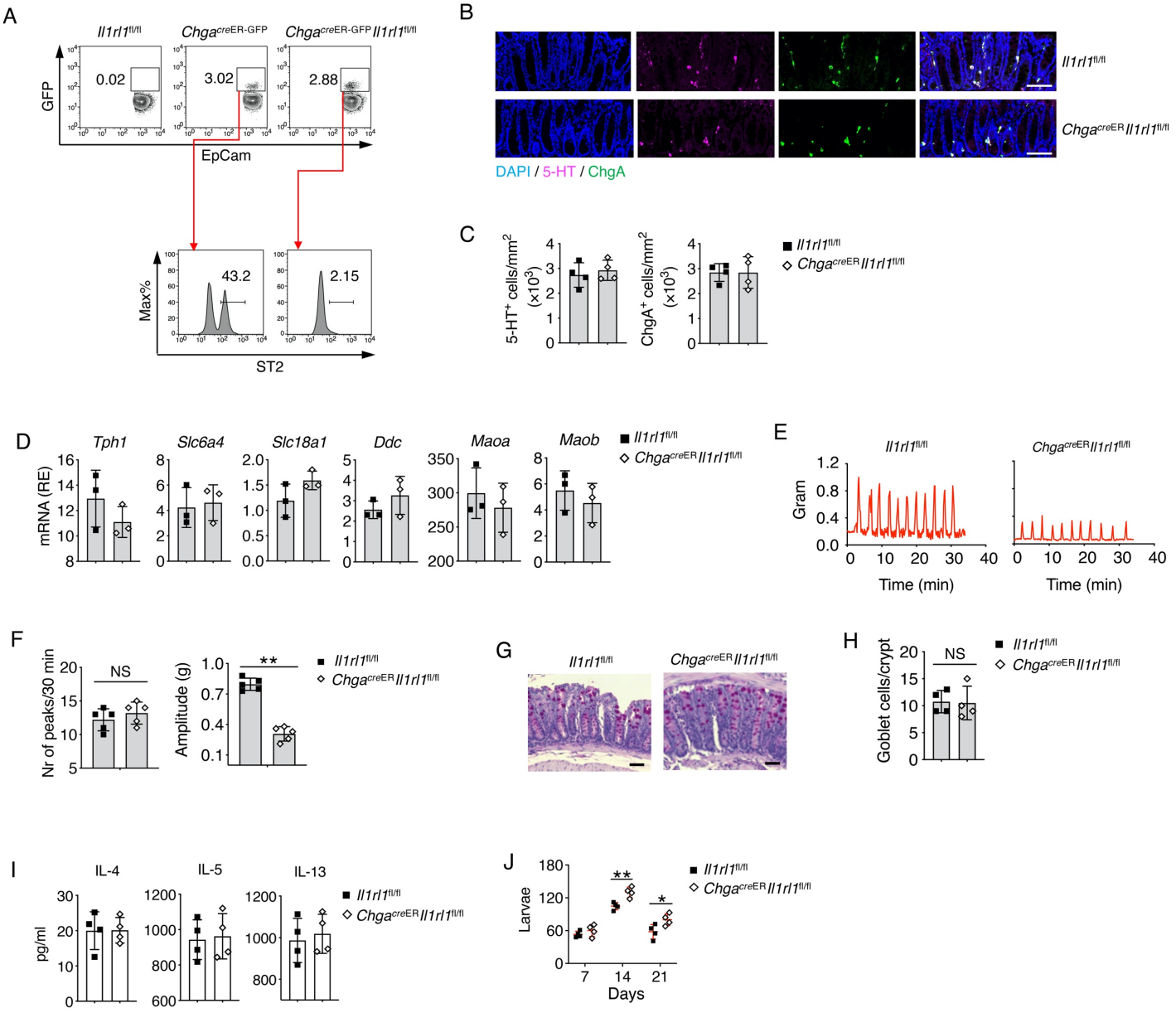

Figure S5. EC cell-derived ST2 responds to IL-33 for 5-HT release, related to Figure 5.

(A) Flow cytometry analysis of IECs isolated from *Il1rl1*$^{fl/fl}$, *Chga*$^{creER\text{-}GFP}$ and *Chga*$^{creER\text{-}GFP}$*Il1rl1*$^{fl/fl}$ mice pretreated with tamoxifen.

(B) Representative images of colons stained for 5-HT, ChgA and DAPI from *Il1rl1*$^{fl/fl}$ and *Chga*^{creER}*Il1rl11*$^{fl/fl}$ mice. Scale bar, 100 μm.

(C) Quantification of 5-HT^{+} and ChgA^{+} cell number per area of colonic tissue in **Figure S5B**.

(D) mRNA levels of indicated genes in IECs from *Il1rl1*$^{fl/fl}$ and *Chga*^{creER}*Il1rl1*$^{fl/fl}$ mice.

(E) Representative trace and (F) Quantification of colon contraction in *Il1rl1*$^{fl/fl}$ and *Chga*^{creER}*Il1rl1*$^{fl/fl}$ mice.

(G) PAS staining and (H) Quantification of goblet cells per crypt of colonic tissues from naïve *Il1rl1*$^{fl/fl}$ and *Chga*$^{Cre ER}$*Il1rl1*$^{fl/fl}$ mice. Scale bar, 50 μm.

(I) IL-4, IL-5 and IL-13 protein levels from mLN of naive *Il1rl1*$^{fl/fl}$ and *Chga*$^{cre ER}$*Il1rl1*$^{fl/fl}$ mice were assessed by ELISA.

(J) *T. muris* eggs were inoculated into *Il1rl1*$^{fl/fl}$ and *Chga*$^{cre ER}$*Il1rl1*$^{fl/fl}$ mice (pre-treated with tamoxifen) by oral gavage and the mice were sacrificed on day 7, 14, 21 to quantify of larvae in the colonic tissues. Data are representative of three independent experiments (A-E, G-J) or are pooled from two independent experiments (F). NS, not significant; *$p < 0.05$; **$p < 0.01$ (Student's *t*-test, error bars represent SD).

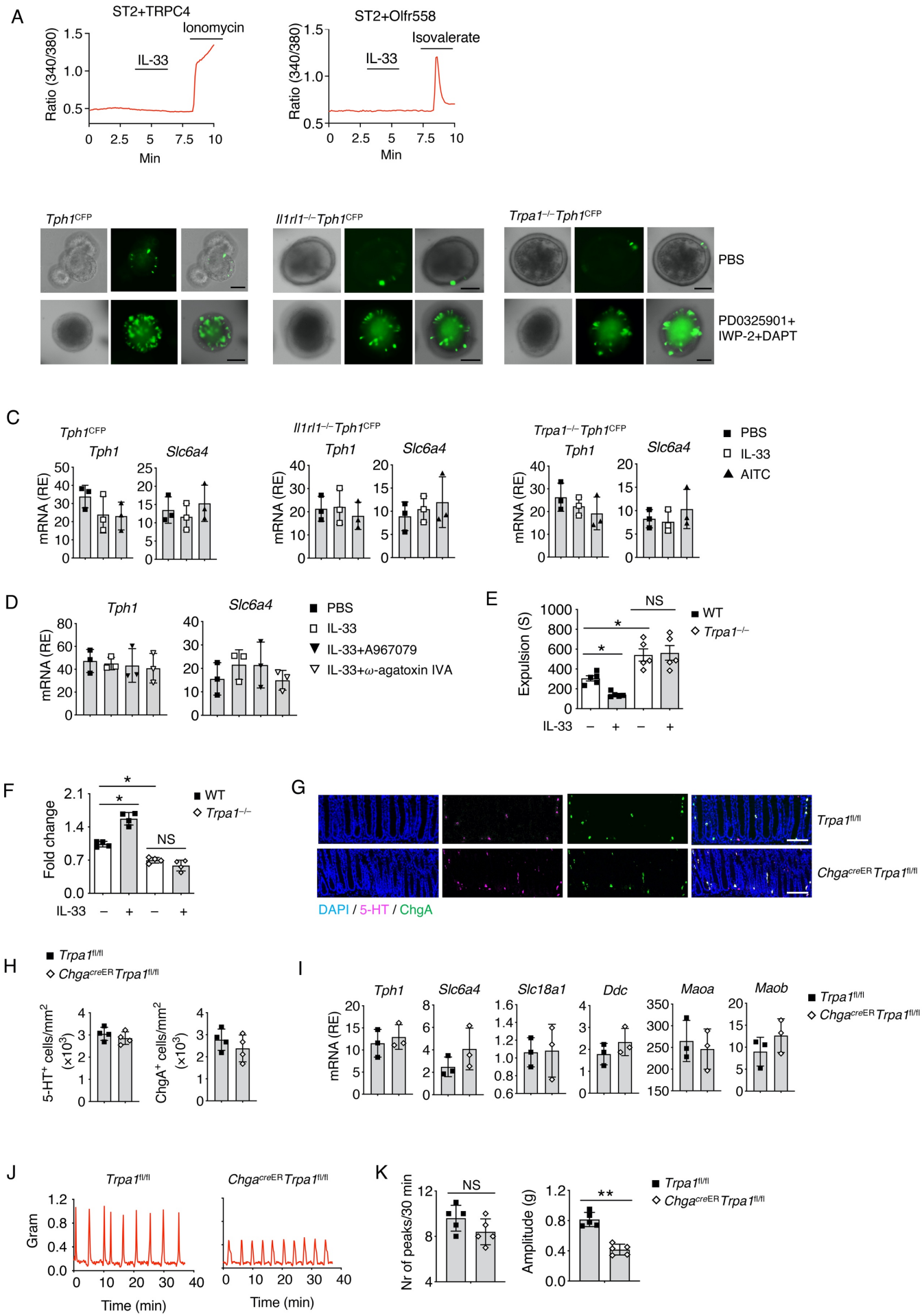
A
ST2+TRPC4
Ionomycin
IL-33
Ratio (340/380)
Min
ST2+Olfr558
Isovalerate
IL-33
Tph1CFP
Il1rl1−/−Tph1CFP
Trpa1−/−Tph1CFP
PBS
PD0325901+
IWP-2+DAPT
C
Tph1
Slc6a4
mRNA (RE)
PBS
IL-33
AITC
D
IL-33+A967079
IL-33+ω-agatoxin IVA
E
Expulsion (S)
NS
WT
Trpa1−/−
IL-33
F
Fold change
G
Trpa1fl/fl
ChgacreERTrpa1fl/fl
DAPI / 5-HT / ChgA
H
5-HT+ cells/mm2 (×103)
ChgA+ cells/mm2 (×103)
I
Slc18a1
Ddc
Maoa
Maob
J
Gram
Time (min)
K
Nr of peaks/30 min
Amplitude (g)

Figure S6. TRPA1 is required for IL-33-mediated 5-HT release, related to Figure 6.

(A) Representative time-lapse Ca^{2+} imaging traces of IL-33-induced $[Ca^{2+}]_i$ response in NIH 3T3 cells overexpressed with ST2 and TRPC4; or with ST2 and Olfr558. Ionomycin and isovalerate were used as positive controls.

(B) TPH1-CFP in the intestinal organoids with or without EC cell-enriched condition from *Tph1*^{CFP}, *Il1rl1*$^{-/-}$*Tph1*^{CFP} and *Trpa1*$^{-/-}$*Tph1*^{CFP} mice. Scale bar, 100 μm.

(C) mRNA levels of *Tph1* and *Slc6a4* from EC-enriched intestinal organoids derived from *Tph1*^{CFP}, *Il1rl1*$^{-/-}$*Tph1*^{CFP} and *Trpa1*$^{-/-}$*Tph1*^{CFP} mice treated with PBS, IL-33 or AITC for 8 hours. AITC (TRPA1 agonist)

(D) mRNA levels of *Tph1* and *Slc6a4* from EC-enriched intestinal organoids derived from WT mice treated with the indicated conditions for 8 hours. A967079 (TRPA1 antagonist). ω-agatoxin IVA (Ca^{2+} channel blocker).

(E-F) WT and *Trpa1*$^{-/-}$ mice were treated with IL-33 or PBS, and waited for 10 minutes. (E) Colonic transit time was assessed by bead expulsion assay. (F) Mice serum was collected, and relative 5-HT levels were assessed by ELISA

(G) Representative images of colons stained for 5-HT, ChgA and DAPI from *Trpa1*$^{fl/fl}$ and *Chga*^{creER}*Trpa1*$^{fl/fl}$ mice. Scale bar, 100 μm.

(H) Quantification of 5-HT$^+$ and ChgA$^+$ cell number per area of colonic tissue in **Figure S6G**.

(I) mRNA levels of indicated genes from IECs of *Trpa1*$^{fl/fl}$ and *Chga*^{creER}*Trpa1*$^{fl/fl}$ mice.

(J) Representative trace and (K) Quantification of colon contraction in *Trpa1*$^{fl/fl}$ and *Chga*^{creER}*Trpa1*$^{fl/fl}$ mice.

Data are representative of three independent experiments (A-D, F-J) or are pooled from two independent experiments (E, K). NS, not significant; *$p < 0.05$; **$p < 0.01$ (Student's *t*-test, error bars represent SD).

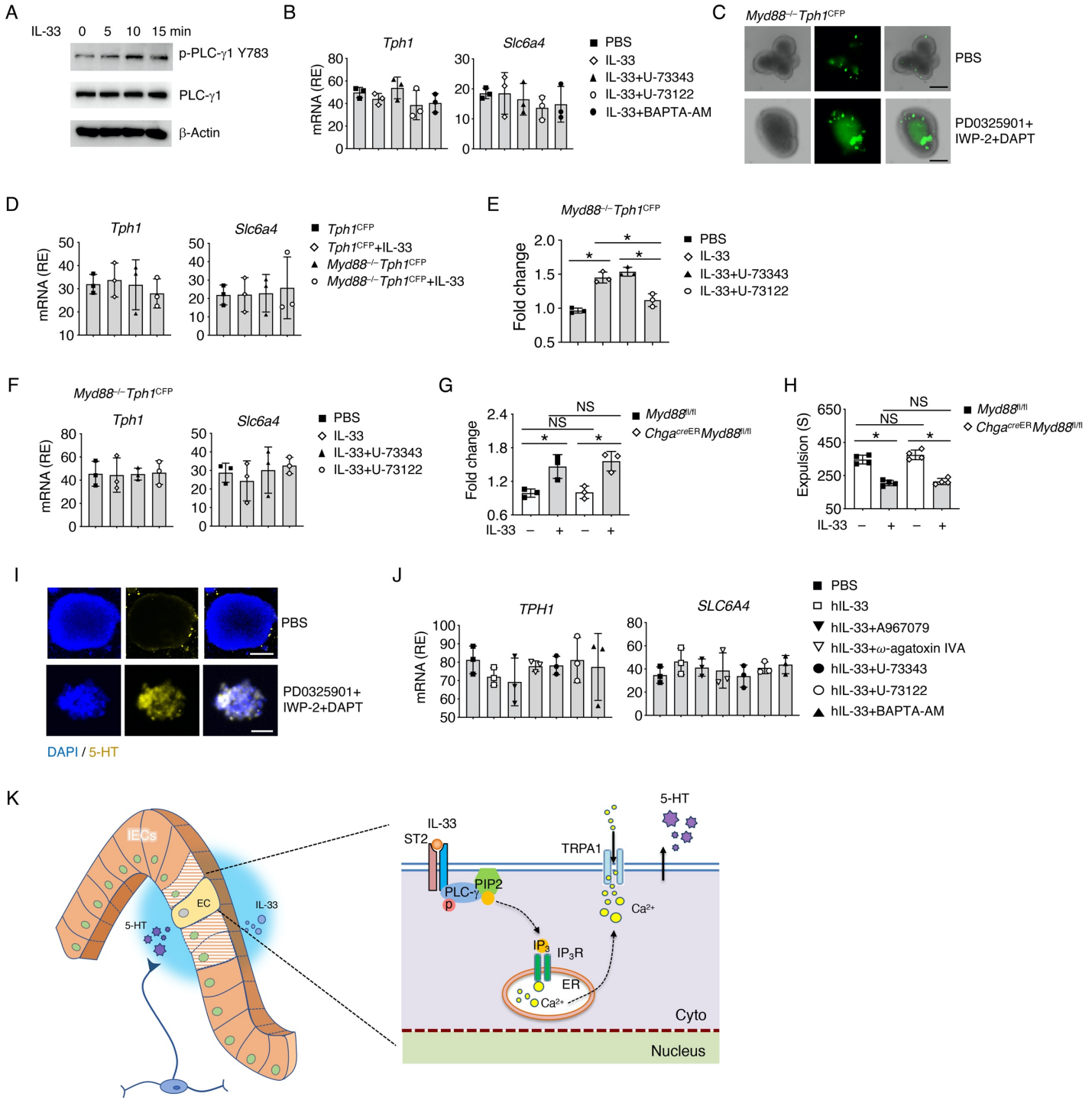

Figure S7. IL-33 induces PLC-γ1 activation for 5-HT release in EC cells, related to Figure 7.

(A) NIH 3T3 cells were transfected with an ST2-expressing vector. Immunoblot analysis of p-PLC-γ1, PLC-γ1 and β-actin in these transfected cells with IL-33 stimulation at the indicated time points.

(B) mRNA levels of *Tph1* and *Slc6a4* from EC-enriched intestinal organoids derived from *Tph1*^{CFP} mice were treated with the indicated conditions for 8 hours. U-73343 (inactive analog of PLC-γ1 inhibitor U-73122). U-73122 (PLC-γ1 inhibitor). BAPTA-AM (calcium chelator, cell-permeant).

(C) TPH1-CFP in the intestinal organoids with or without EC cell-enriched conditions from *Myd88*$^{-/-}$ *Tph1*^{CFP} mice. Scale bar, 100 μm.

(D) mRNA levels of *Tph1* and *Slc6a4* from EC-enriched intestinal organoids derived from *Tph1*^{CFP} and *Myd88*$^{-/-}$*Tph1*^{CFP} mice treated with or without IL-33 for 8 hours.

(E) Relative 5-HT levels and (F) mRNA levels of *Tph1* and *Slc6a4* from in EC-enriched intestinal organoids derived from *Myd88*$^{-/-}$*Tph1*^{CFP} mice treated with the indicated conditions for 8 hours were assessed.

(G-H) *Myd88*$^{fl/fl}$ and *Chga*$^{cre ER}$*Myd88*$^{fl/fl}$ mice were treated with PBS or IL-33, and waited for 10 minutes. (G) Mice serum was collected, and relative 5-HT levels were assessed by ELISA. (H) Colonic transit time was assessed by bead expulsion assay.

(I) Representative immunofluorescence staining for 5-HT with DAPI in the human iPSC-derived intestinal organoids with or without EC-enriched conditions. Scale bar, 100 μm.

(J) mRNA levels of *TPH1* and *SLC6A4* in EC-enriched human intestinal organoids derived from human iPSC cells treated with the indicated conditions for 8 hours.

(K) Proposed model for how the IL-33-ST2 axis promotes 5-HT release in EC cells.

Data are representative of three independent experiments (A-J). NS, not significant; *$p < 0.05$; **$p < 0.01$ (Student's *t*-test, error bars represent SD).