

Figure S1

Figure S1. Tape stripping causes expansion of intestinal submucosal and mucosal MCs and of intestinal MCs in different mice strains and independently of the microbiota, related to Figure 1. **A.** Representative immunohistochemistry staining for mMCPT1 (top) and mMCPT6 (bottom) of jejunal sections from tape stripped (T/S) mice and unmanipulated (Unm.) controls. **B.** Absolute numbers of c-kit⁺IgE⁺ MCs in duodenum (left) and colon (right) of T/S mice and Unm. controls. Results are derived from 2 independent experiments with 3 to 5 mice/group. **C.** Numbers of CD45⁺ cells in the jejunum of T/S mice and Unm. controls. Results are derived from 2 independent experiments with 3 to 5 mice/group. **D.** Absolute numbers of jejunal c-kit⁺IgE⁺ MCs in T/S and Unm. BALB/c (left) and C57BL/6 (right) mice from Charles River Labs (CRL) kept at Children's Hospital Boston specific pathogen free (SPF) facility. Data representative of 2 independent experiments each with 3 to 4 mice/group. **E.** Absolute numbers of jejunal c-kit⁺IgE⁺ MCs in T/S and Unm. BALB/c mice from Taconic Biosciences (Tac) kept at Children's Hospital Boston SPF (left) or germ free (GF) facility. Data representative of 2 independent experiments each with 3 to 4 mice/group. Columns and bars represent mean and SEM. ** = p < 0.01, *** = p < 0.001, ns: not significant.

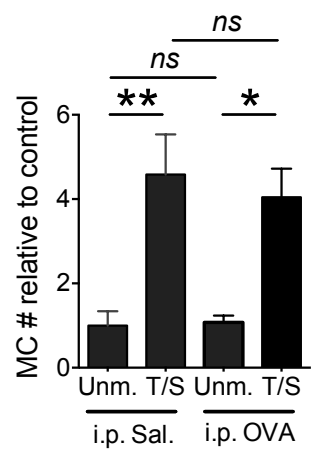
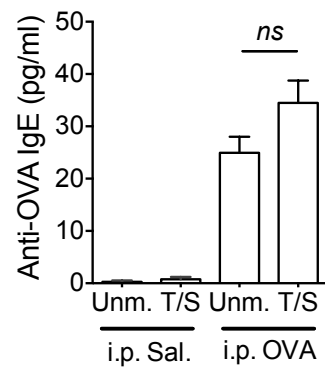
A**B**

Figure S2. Intestinal MC expansion and antigen specific IgE in mice tape stripped and i.p. immunized with OVA, related to Figure 2. A-B. Relative numbers (#) of jejunal MCs (A) and Serum anti-OVA IgE antibody concentrations (B) in WT BALB/c mice *i.p.* immunized with OVA or saline and tape stripped (T/S) or left unmanipulated (Unm.) as per the protocol in Fig. 2B. Data representative of 2 independent experiments each with 4-5 mice/group. Columns and bars represent mean and SEM. * = $p < 0.05$, ** = $p < 0.01$, ns: not significant.

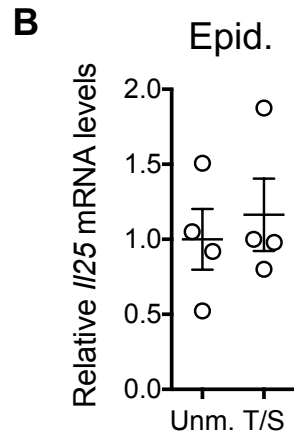
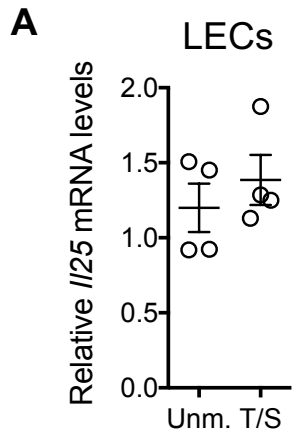


Figure S3. Effect of tape stripping on *I/25* mRNA expression in lung and skin epithelial cells, related to Figure 3. A. *I/25* mRNA expression in lung epithelium cells (LECs) and skin epidermal layer (Epid.) from ears of mice tape stripped on back skin or unmanipulated controls. ns: not significant. Values represent fold induction relative to the mean value in unmanipulated mice. Results are derived from 2 independent experiments each with respectively 3 -5 mice/group.

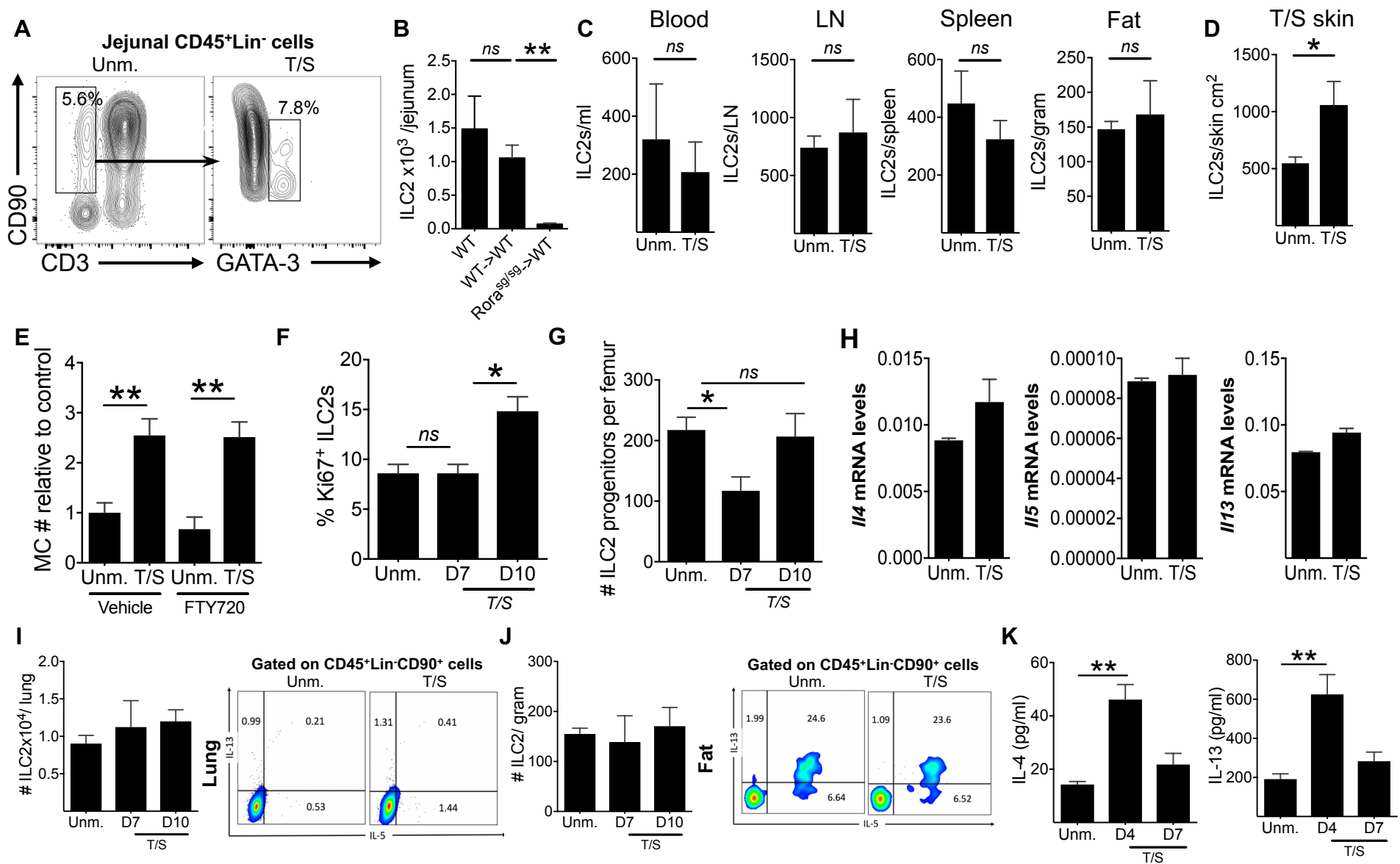


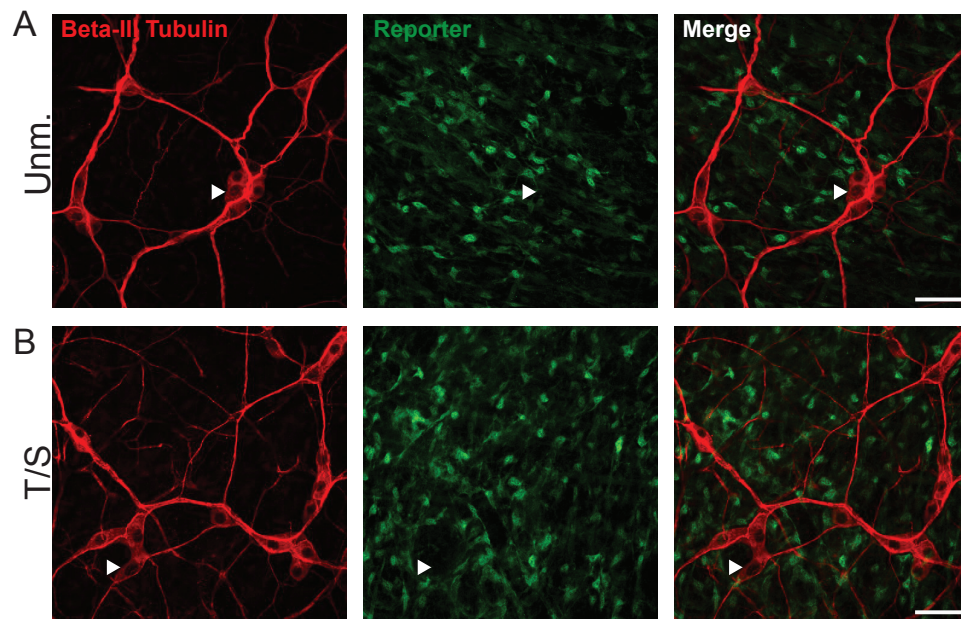
Figure S4

Figure S4. Gating strategy for intestinal ILC2s, absence of ILC2s in the intestine of *Rora*^{sg/sg}->WT, number of ILC2s in different tissues, effect of the sphingosine-1-phosphate (S1P) analogue FTY720 on intestinal MC expansion, effect of tape stripping on proliferation of jejunal ILC2s and mobilization of ILC2 progenitors from the bone marrow, type 2 cytokine expression in ILCs from distant skin sites, lungs and fat and Serum IL-4 and IL-13 are increased by tape stripping prior to intestinal ILC2 expansion related to Figure 4.

A. Representative flow cytometry analysis of CD90⁺CD3⁻GATA-3⁺ ILC2s in the CD45⁺Lin⁻ fraction of LP cells from the jejunum. **B.** Numbers of jejunal ILC2s in unmanipulated *Rora*^{sg/sg}->WT chimeras, WT-WT chimeras and WT mice controls. **C-D.** Numbers of CD45⁺Lin⁻CD90⁺GATA3⁺ ILC2s in blood, lymph node (LN), spleen, visceral adipose tissue (C) and in the unmanipulated and tape stripped skin (D) of unmanipulated (Unm) and tape stripped (T/S) WT BALB/c mice. **E.** Jejunal MC numbers (#) in unmanipulated (Unm) and tape stripped (T/S) BALB/c mice treated with FTY720 or vehicle. **F.** Percentage of CD45⁺Lin⁻CD90⁺GATA3⁺Ki67⁺ cells in the jejunum of unmanipulated (Unm) and tape stripped (T/S) WT mice. **G.** Numbers (right) of Lin⁻Sca1⁺IL-25R⁺CD25⁺CD127⁺ ILC2 progenitors (ILC2p) in bone marrow cells from T/S or Unm. mice. **H.** *Il4*, *Il5* and *Il13* mRNA expression, relative to *B2m*, in sorted CD45⁺Lin⁻CD90⁺ ILCs from distant skin sites of T/S mice and skin from Unm. controls. Data pooled from 2 independent experiments each with 3-4 mice/group. Columns and bars represent mean and SEM. **I-J.** ILC2 quantitation in lungs and visceral adipose tissue of WT BALB/c mice mice on Days 0, 7 and 10 post tape stripping (left panels) and representative flow cytometry analysis of the expression of IL-5 and IL-13 by CD45⁺Lin⁻CD90⁺ ILCs in the lungs and visceral adipose tissue of Unmanipulated (Unm) mice and mice on Day 7 after tape stripping (right panels).

K. Serum concentration of IL-4 (left) and IL-13 (right) in tape stripped (T/S) or left unmanipulated (Unm.) WT mice at different time points. Results are derived from 2 independent experiments.

Submucosal Plexus



Myenteric Plexus

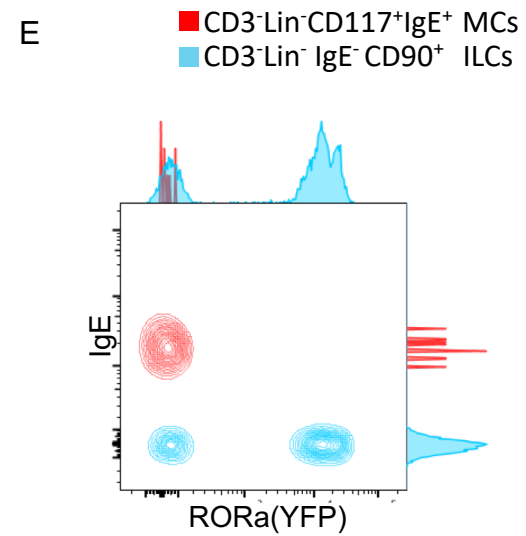
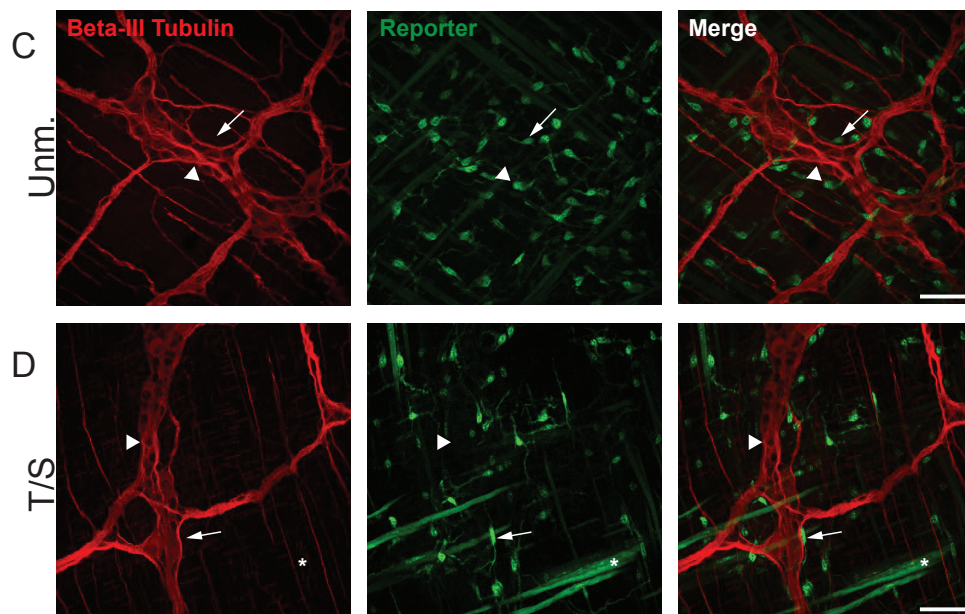


Figure S5

Figure S5. ROR α is not expressed in intestinal neurons or MCs, related to Figure 5.A-

D. Representative images from jejunum of tape stripped unmanipulated (Unm) and tape stripped (T/S) *Rora*^{cre/cre}*ROSA*^{YFP} mice. Beta-III tubulin is a pan-neuronal marker, labeling neuron bodies and processes. In the submucosal plexus, YFP expression rarely, if ever, overlapped with Beta-III tubulin expression in (A) T/S mouse submucosal plexus vs (B) Unm. mouse submucosal plexus (arrowheads). Similar to the submucosal plexus, reporter expression very rarely overlapped with Beta-II tubulin expression in (A) T/S mouse vs (B) Unm. mouse (arrowheads). Reporter expression was seen in cells with smooth muscle morphology (asteriks) as well as cells near the periphery of ganglia, possibly interstitial cells of Cajal, glia, or immune cells based on location, morphology and processes (arrows). 40x. Scale bar = 50 μ m. **E.** Representative flow cytometry analysis of *Rora* (YFP) expression in intestinal ILCs (CD45⁺Lin⁻CD90⁺) and MCs (CD45⁺Lin⁻CD117⁺ α -IgE⁺) in *Rora*^{cre/cre}*ROSA*^{YFP} mice.

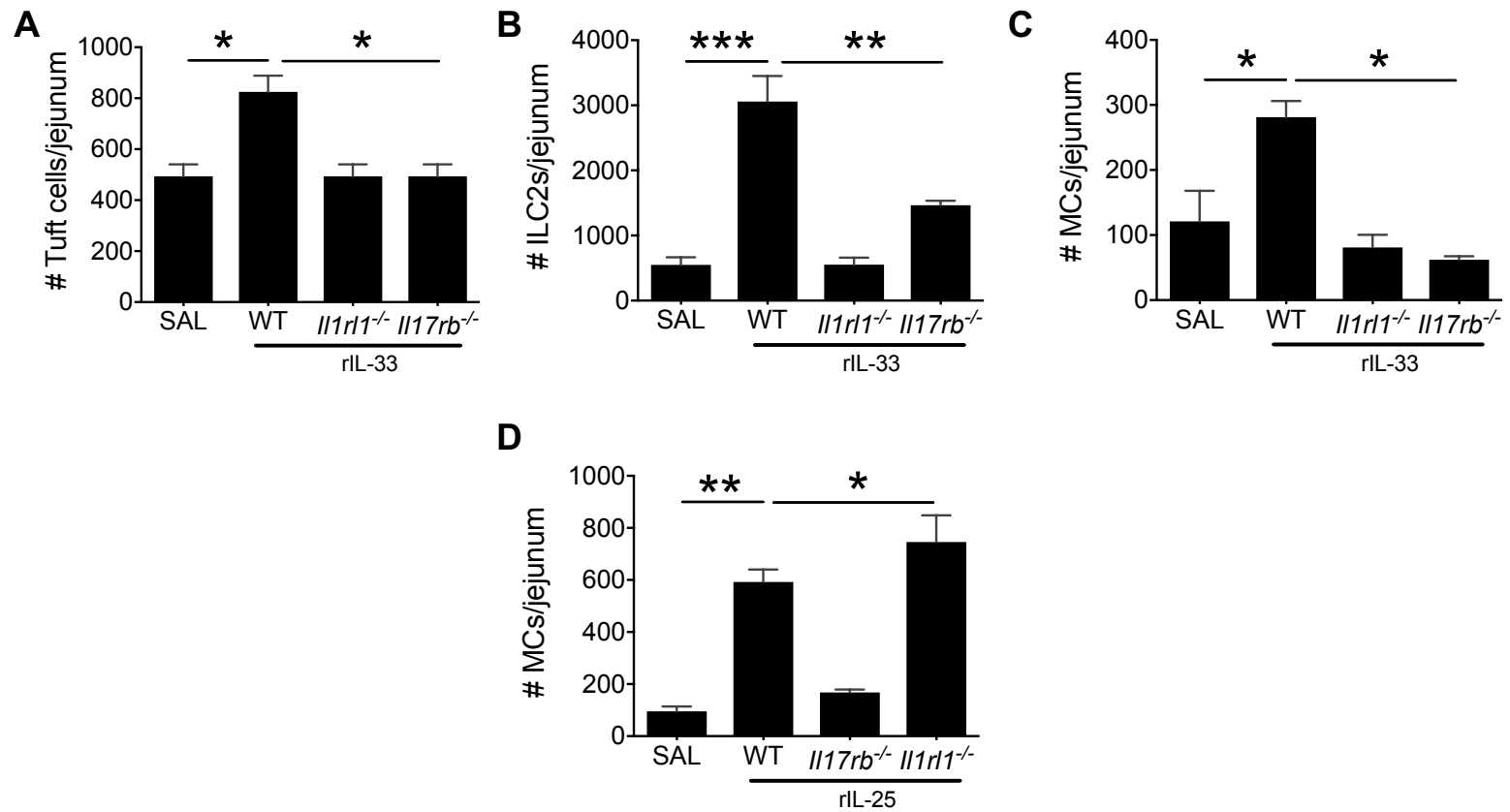


Figure S6

Figure S6. Effect of i.v injection of rIL-33 and rIL-25 in the expansion of intestinal tuft cells, ILC2 and MCs, related to Figure 5. A-C. Number of CD45⁻SiglecF⁺EPCAM⁺ tuft cells (A), CD45⁺Lin⁻CD90⁺GATA3⁺ ILC2s (B) and CD45⁺Lin⁻CD117⁺ α -IgE⁺ MCs (C) in the jejunum of *Il1rl1*^{-/-} and *Il17rb*^{-/-} mice and WT controls injected with rIL-33. **D.** Number of MCs in the jejunum of *Il17rb*^{-/-} and *Il1rl1*^{-/-} mice and WT controls injected with rIL-25. Results are derived from 2 independent experiments with 3 to 5 mice/group. Columns and bars represent mean and SEM. * = p < 0.05, ** = p < 0.01, *** = p < 0.001.