## Full Title: **TSLP-mediated epicutaneous inflammation promotes acute diarrhea and anaphylaxis**

Condensed Title: TSLP Links Atopic Dermatitis to Gastrointestinal Allergy

Authors: Hongwei Han<sup>1</sup>, Tennille D. Thelen<sup>1,2</sup>, Michael R. Comeau<sup>3</sup>, Steven F. Ziegler<sup>1,2</sup>

- <sup>1</sup> Immunology Program, Benaroya Research Institute, Seattle, Washington 98101, USA
- <sup>2</sup> Department of Immunology, University of Washington School of Medicine, Seattle, Washington 98195, USA
- <sup>3</sup> Inflammation Research, Amgen Inc., Seattle, Washington 98119, USA



**Supplemental Figure 1.** Representative jejunum tissue cross sections stained with hematoxylin and eosin (H&E) to visualize eosinophils (arrow). Bar: 100 µm.



MSA+OVA

TSLP+OVA

**Supplemental Figure 2.** Representative histology of tissue stained with H&E demonstrating a robust eosinophilic infiltration into the jejunum tissues. Yellow arrows indicate eosinophils. Bar: 100 µm.



**Supplemental Figure 3.** Gastrointestinal allergy develops from mice rested 21 days. (A) Diarrhea occurrence. (B) Diarrhea score. (C) OVA-specific serum IgE levels. (D) MMCP-1 serum levels. The significance between two groups was determined by two-tailed Student's t test. (E) Intracellular cytokine staining of MLN cells. Plots are gated on CD4<sup>+</sup>CD44<sup>hi</sup> cells and representative of four mice analyzed. For A, C and D, data are representative of two independent experiments with four mice per group. Error bars indicate the mean ± SD.



**Supplemental Figure 4.** Intradermal administration of TSLP and whole peanut extract (WPE) promotes gastrointestinal allergy. (A) Diarrhea occurrence. (B) Diarrhea score. (C) MMCP-1 serum levels. (D) WPE-specific serum IgE levels. (E) TSLP serum levels. N.D., <7.8 pg/ml. Data are representative of two independent experiments with four mice per group. Error bars indicate the mean ± SD.



**Supplemental Figure 5.** Generation and analysis of TSLPR conditional mutant mice. (A) Diagrams of the TSLPR targeting construct, the genomic locus (TSLPR<sup>+</sup>) and the neomycin-containing (TSLPR<sup>neo</sup>), the *loxP*-flanked (TSLPR<sup>lox</sup>) and the null (TSLPR<sup>-</sup>) TSLPR allels. Filled black boxes, exons; red boxes, *loxP* (L) sites; green boxes, FRT (F) sites. (B) Flow cytometry analysis of TSLPR in splenic dendritic cells. Plots are gated on CD11c<sup>+</sup>MHCII<sup>hi</sup> cells and representative of 3 mice analyzed. (C) MFI of TSLPR on the CD11c<sup>+</sup>MHCII<sup>hi</sup> DCs. Data are representative of four independent experiments with three mice per group. Error bars indicate the mean ± SD.



**Supplemental Figure 6.** Basophil responses are required in gastrointestinal allergy. (A) Frequencies of basophils in the spleen. Plots are gated on  $CD3^{-}CD19^{-}Siglec-F^{-}CD49b^{+}IgE^{+}$  cells and representative of 4 mice analyzed. (B and C) Serum leakage in the intestine. The representative images (B) and quantified graph of leaked dye (C) are indicated. Error bars indicate the mean  $\pm$  SD.



**Supplemental Figure 7.** TSLPR on DCs is required for epicutaneous priming. (A) Cell number in inguinal lymph nodes (ILN) at D15. (B) OVA-specific serum IgE levels at D15. Data are representative of two independent experiments with four mice per group. Error bars indicate the mean ± SD.