

## Supplementary Materials and Methods

*Loss of DSG1 expression in CC10-ill13 bitransgenic mice.* Generation of the *CC10-ill13* bitransgenic mice and induction of experimental EoE has been previously described<sup>1</sup>. Untreated or doxycycline-treated mice were sacrificed 30 days post treatment. IL-13 levels from the BAL were assessed by ELISA (R&D Systems, Minneapolis, MN). Esophageal eosinophilia was detected by immunohistochemical staining for major basic protein (MBP) expression as described<sup>1</sup>. Expression of *Dsg1* ( $\alpha$ ,  $\beta$ , and  $\gamma$ ) and the housekeeping gene *Gapdh* was assessed by qPCR as described in the Materials and Methods using the following primers: *Dsg1* (ATGTCTGCAGAGAGCGAGTG and CCAATTCCTCATTCCAG); *Gapdh* (CGGGAAGCCCATCACCATCT and GTCTTCTGGGTGGCAGTGAT). Detection of DSG1 protein by immunofluorescent staining of mouse esophageal sections was performed as described in the Materials and Methods; note the anti-DSG1 antibody used (sc-2011) also cross-reacts with mouse DSG1 and does not discriminate between the various DSG1 isoforms. Data were generated from a total of 19 mice (untreated [- Dox] = 8, treated [+ Dox] = 11) from two independent experiments. All animals were housed under specific pathogen-free conditions in accordance with Institutional Animal Care and Use Committee (IACUC) guidelines.

## References

1. Zuo L, Fulkerson PC, Finkelman FD, Mingler M, Fischetti CA, Blanchard C *et al.* IL-13 induces esophageal remodeling and gene expression by an eosinophil-independent, IL-13R alpha 2-inhibited pathway. *J Immunol* 2010; **185**(1): 660-669.

Supplementary Table 1. Fold change in expression of overlapping dysregulated transcripts (n = 32)

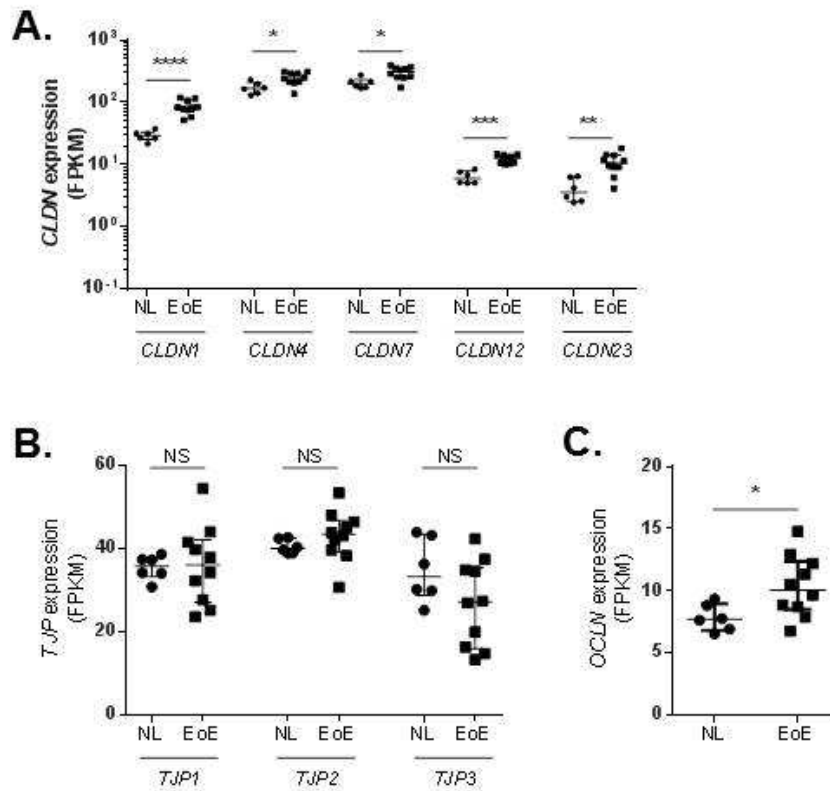
Gene symbol	Fold change	
	ALI microarray DSG1 vs. NSC shRNA	Esophageal biopsy RNA-seq EoE vs. NL
<i>POSTN</i>	2.50	383.84
<i>SECTM1</i>	-2.34	17.99
<i>CAPN14</i>	-2.19	5.58
<i>HJURP</i>	2.12	3.26
<i>BUB1</i>	2.30	3.03
<i>DLGAP5</i>	2.45	2.97
<i>CENPE</i>	2.19	2.97
<i>HMMR</i>	2.11	2.97
<i>ANLN</i>	2.07	2.91
<i>ASPM</i>	2.24	2.86
<i>TTK</i>	2.07	2.74
<i>KIF20A</i>	3.13	2.73
<i>PBK</i>	2.13	2.68
<i>NDC80</i>	2.15	2.68
<i>KIF18A</i>	2.19	2.57
<i>TPX2</i>	2.30	2.52
<i>CCNA2</i>	2.28	2.51
<i>PLK1</i>	2.50	2.47
<i>CKS2</i>	2.04	2.38
<i>ARHGAP11A</i>	2.21	2.36
<i>NCAPG</i>	2.21	2.35
<i>ARHGAP11B</i>	2.03	2.11
<i>CRISP3</i>	-3.20	-89.35
<i>MUC21</i>	-2.25	-13.21
<i>DSG1</i>	-3.28	-12.86
<i>ENDOU</i>	-2.14	-9.14
<i>ZNF812</i>	-2.49	-8.35
<i>MAL</i>	-2.41	-3.24
<i>TMPRSS11B</i>	-2.29	-3.11
<i>CXCL14</i>	3.44	-2.86
<i>CAPN5</i>	-2.38	-2.79
<i>SRPX2</i>	-2.09	-2.09

Supplementary Table 2. Quantitative PCR primer sequences

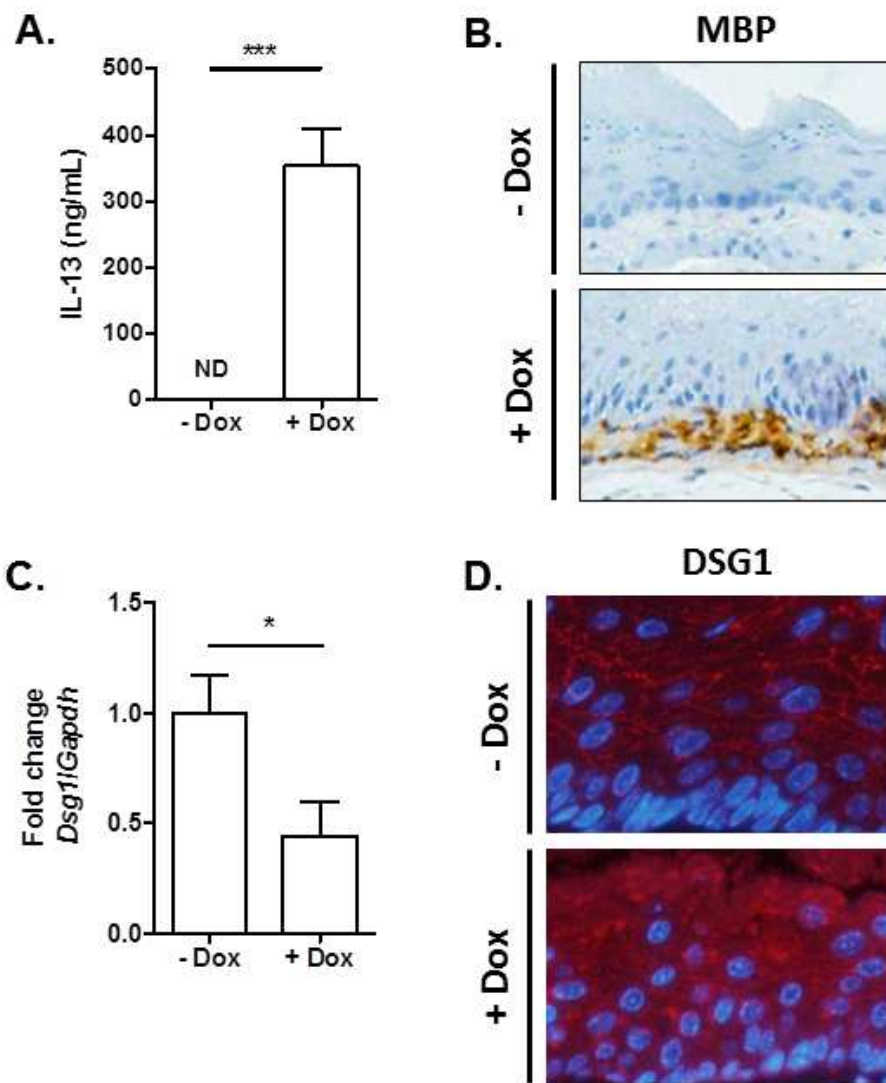
<b>Gene symbol</b>	<b>Forward primer (5' to 3')</b>	<b>Reverse primer (5' to 3')</b>
<i>DSG1</i>	TGGAGAAATTCGAACGATGA	CACTCACATTCCGCTGACA
<i>DSG3</i>	TCTGGAACCATGAGAACAAGG	CACAGGCAAATGCTTTCTGA
<i>POSTN</i>	AGATCCGTGAAGGTGGTTTG	GTCTTTGAGACGCTGGAAGG
<i>KRT10</i>	AGCATGGCAACTCACATCAG	TGTCGATCTGAAGCAGGATG
<i>GAPDH</i>	TGGAAATCCCATCACCATCT	GTCTTCTGGGTGGCAGTGAT

**Supplementary Table 3. Effects of *DSG1* knockdown and IL-13 on barrier genes**

			<b>ALI microarray</b>			
			<b><u>DSG1 vs. NSC shRNA</u></b>		<b><u>IL-13 (100 ng/mL) vs. untreated</u></b>	
<b><u>Transcript Cluster Id</u></b>	<b><u>Gene symbol</u></b>	<b><u>Gene name</u></b>	<b><u>p-value</u></b>	<b><u>Fold change</u></b>	<b><u>p-value</u></b>	<b><u>Fold change</u></b>
<a href="#"><u>16820486</u></a>	<a href="#"><u>CDH1</u></a>	<a href="#"><u>cadherin 1, type 1, E-cadherin (epithelial)</u></a>	<a href="#"><u>0.20</u></a>	<a href="#"><u>-1.06</u></a>	<a href="#"><u>0.20</u></a>	<a href="#"><u>-1.08</u></a>
<a href="#"><u>16962661</u></a>	<a href="#"><u>CLDN1</u></a>	<a href="#"><u>claudin 1</u></a>	<a href="#"><u>0.69</u></a>	<a href="#"><u>-1.01</u></a>	<a href="#"><u>0.16</u></a>	<a href="#"><u>1.09</u></a>
<a href="#"><u>17046982</u></a>	<a href="#"><u>CLDN4</u></a>	<a href="#"><u>claudin 4</u></a>	<a href="#"><u>0.05</u></a>	<a href="#"><u>-1.08</u></a>	<a href="#"><u>0.01</u></a>	<a href="#"><u>-1.23</u></a>
<a href="#"><u>16985704</u></a>	<a href="#"><u>OCLN</u></a>	<a href="#"><u>occludin</u></a>	<a href="#"><u>0.43</u></a>	<a href="#"><u>-1.06</u></a>	<a href="#"><u>0.03</u></a>	<a href="#"><u>-1.38</u></a>
<a href="#"><u>16806467</u></a>	<a href="#"><u>TJP1</u></a>	<a href="#"><u>tight junction protein 1 (zona occludens 1)</u></a>	<a href="#"><u>0.06</u></a>	<a href="#"><u>-1.05</u></a>	<a href="#"><u>0.03</u></a>	<a href="#"><u>-1.13</u></a>



**Supplementary Figure 1. Tight junction gene expression in EoE.** Expression levels of claudins (*CLDN*) (A), tight junction proteins (*TJP*) (B), and occludin (*OCLN*) (C) from RNA sequencing of esophageal biopsies from 10 patients with active EoE and 6 healthy controls (NL). Only those genes with detectable levels of expression (FPKM > 1) in all samples are shown. Data are represented as the median + interquartile range: NS (not significant), \*  $p < 5 \times 10^{-2}$ , \*\*  $p < 5 \times 10^{-3}$ , \*\*\*  $p < 5 \times 10^{-4}$ , and \*\*\*\*  $p < 1 \times 10^{-4}$ .



**Supplementary Figure 42. IL-13 downregulates murine esophageal expression of DSG1 in vivo.**

Levels of IL-13 in the BAL of CC10-*ill13* transgenic mice treated with (+ Dox) or without (- Dox) doxycycline (A). Immunohistochemical staining of esophageal sections from CC10-*ill13* transgenic mice for the eosinophil granule protein, major basic protein (MBP) (B). qPCR analysis (C) and immunofluorescence staining for DSG1 (D) in the esophageal mucosa of CC10-*ill13* bitransgenic mice. Nuclei are stained with DAPI (blue). Data are derived from two independent experiments (untreated (- Dox)  $n = 8$  and treated (+ Dox)  $n = 11$ ) and are represented as the mean + SEM: ND not detected, \*  $p < 5 \times 10^{-2}$ , \*\*  $p < 5 \times 10^{-3}$ , and \*\*\*  $p < 1 \times 10^{-3}$ .



**LICENSE TO PUBLISH**

**Please FAX this completed form to +1 508-276-0594, Attention: Jason Roberts**

**Manuscript #:** MI-13-187 ..... **Mucosal Immunology**

**Title of the contribution:** Desmoglein-1 regulates esophageal epithelial barrier function .....  
("the Contribution")

and immune responses in eosinophilic esophagitis .....  
**Author(s):** (names only) Joseph D. Sherrill, Kiran KC, David Wu, Zorka Djukic, Julie M. Caldwell, Emily M. Stucke, Katherine A. Kemme, Mark S. Costello, Melissa K. Mingler, Carine Blanchard, Margaret H. Collins, J. Pablo Abonla, Philip E. Putnam, Evan S. Dellon, Roy C. Orlando, Simon P. Hogan, Marc E. Rothenberg .....  
("the Authors")

**To: The Society for Mucosal Immunology ("SMI")**

1. In consideration of SMI agreeing to publish the Contribution the Authors grant to SMI for the full term of copyright in the Contribution and any extensions thereto, subject to clause 2 below, the exclusive license (a) to publish, reproduce, distribute, display and store the Contribution in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Contribution into other languages, create adaptations, summaries or extracts of the Contribution or other derivative works based on the Contribution and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts and derivative works and (c) to license others to do any or all of the above.
2. Ownership of copyright remains with the Authors, and provided that, when reproducing the Contribution or extracts from it, the Authors acknowledge first and reference publication in the Journal, the Authors retain the following non-exclusive rights:
  - a) To reproduce the Contribution in whole or in part in any printed volume (book or thesis) of which they are the author(s).
  - b) They and any academic institution where they work at the time may reproduce the Contribution for the purpose of course teaching.
  - c) To post a copy of the Contribution as accepted for publication after peer review (in Word or Tex format) on the Author's own web site, or the Author's institutional repository, or the Author's funding body's archive, six months after publication of the printed or online edition of the Journal, provided that they also link to the Journal article on NPG's web site (eg through the DOI).
  - d) To reuse figures or tables created by them and contained in the Contribution in other works created by them.

3. The Authors warrant and represent that:

- a) The Authors are the sole authors of and sole owners of the copyright in the Contribution. If the Contribution includes materials of others, the Authors have obtained the permission of the owners of the copyright in all such materials to enable them to grant the rights contained herein. Copies of all such permissions are attached to this license.
  - b) All of the facts contained in the Contribution are true and accurate.
  - c) The Author who has signed this Agreement has full right, power and authority to enter into this Agreement on behalf of all of the Authors.
  - d) Nothing in the Contribution is obscene, defamatory, libelous, violates any right of privacy or infringes any Intellectual property rights (including without limitation copyright, patent or trademark) or any other human, personal or other rights of any person or entity or is otherwise unlawful.
  - e) Nothing in the Contribution infringes any duty of confidentiality which any of the Authors may owe to anyone else or violates any contract, express or implied, of any of the Authors, and all of the institutions in which work recorded in the Contribution was carried out have authorized publication of the Contribution.
4. The Authors authorize SMI to take such steps as it considers necessary at its own expense in the Authors' name and on their behalf if it believes that a third party is infringing or is likely to infringe copyright in the Contribution.

This Agreement and the rights and liabilities of the parties with respect to this Agreement and its subject matter, shall be governed by the laws of the State of New York, without reference to the principles of conflicts of laws thereof.

**Signed for and on behalf of the Authors:** *Marc E. Rothenberg* ..... **Date:** July 18, 2013

**Please print name:** Marc E. Rothenberg, M.D., Ph.D. ....  
**Address:** Cincinnati Children's Hospital Medical Center, Division of Allergy and Immunology .....  
3333 Burnet Avenue, M.L.C. 7028, Cincinnati, OH 45229

# ARTWORK AND SUPPLEMENTARY INFORMATION PRODUCTION FORM

This form must be completed for all papers. We are unable to process your paper until we receive instructions concerning artwork and supplementary files.

Please return this form to via fax to: + 1 508 276-0594

Journal: **Mucosal Immunology**

Article Title: Desmoglein-1 regulates esophageal epithelial barrier function and immune responses in eosinophilic esophagitis

Manuscript Number: MI-13-187

## A. Does Your Article Have Color Artwork for Print Publication?

No.

Yes. I agree to pay the color charges in full and hereby authorize Nature Publishing Group to invoice me for the cost of reproducing color artwork in print. There is no charge for publishing black and white figures.

Note: The cost for reproducing color artwork in print is \$613 per page. Color figures will be set close to the citation and in the best possible position. Figure re-ordering cannot be undertaken at proof stage to reduce the number of pages featuring color artwork.

Which figures? (e.g. Figures 1a, 2, 3b)

Figures 1a, 2, 3a, 4c, 4e, 4d, 6a, 7a, 9

## B. Do You Have Figures for Reproduction in Color on the Online Full Text HTML Version Only?

No.

Yes. I am supplying figures for color reproduction for the online full text HTML version. These figures will also be supplied as separate black and white files for the print and online PDF versions.

Note: There is no charge for reproducing color figures for the online full text HTML version published online. Please note that color will appear in the full text HTML version only, and not in the online PDF version.

Which figures? (e.g. Figures 1c, 5, 6)

## C. Does Your Article Have Supplementary (online-only) Information?

No.

Yes. I agree to pay the Supplementary Information charges in full and hereby authorize Nature Publishing Group to invoice me for the amount below.

Note: Supplementary Information is charged at \$125.00 for each file.

1 file(s) x \$125.00 Total amount: \$125.00

What material is supplied as Supplementary Information? Supplementary Materials and Methods, Supplementary Tables 1-3, and Supplementary Figures 1 and 2

Signature: Marc Rothenberg

Print Name: Marc E. Rothenberg, MD, PhD

Date: 9/17/2013

2/11/JM/US/MI