IL-33 in asthma: From farming communities to animal models

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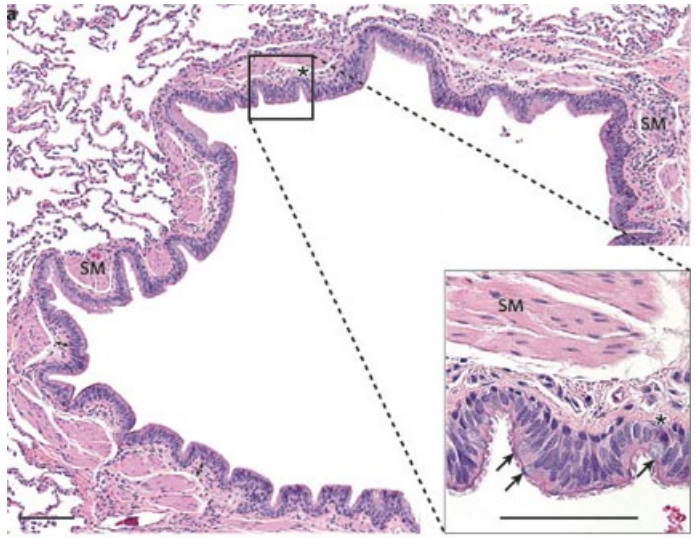
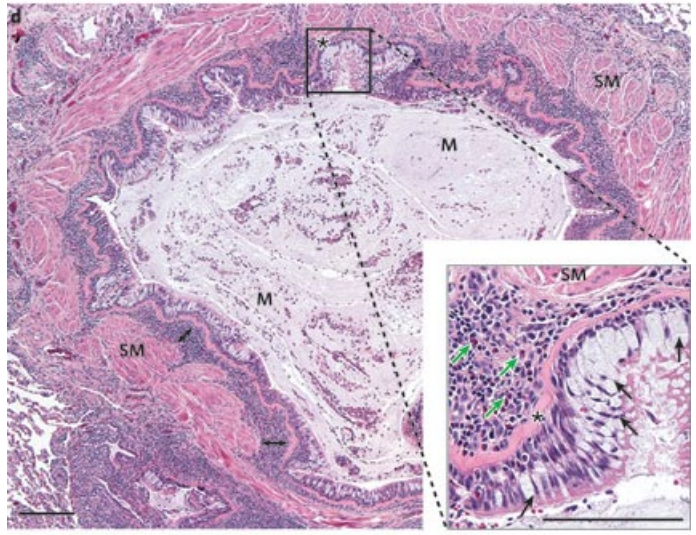
No Conflicts of Interest

Allergic asthma (Th2) is a chronic inflammatory disease of the airways

- Chronic inflammatory disease of the lung, affects over 300 million people ~7.5% of adult population (Braman Chest 2006, McCracken JAMA 2017)
- Symptoms: shortness of breath, chest tightness, and bouts of coughing or wheezing
- Pathology
  - Inflammation
  - Goblet cell mucus production
  - Smooth muscle thickening
  - Reversible airway constriction



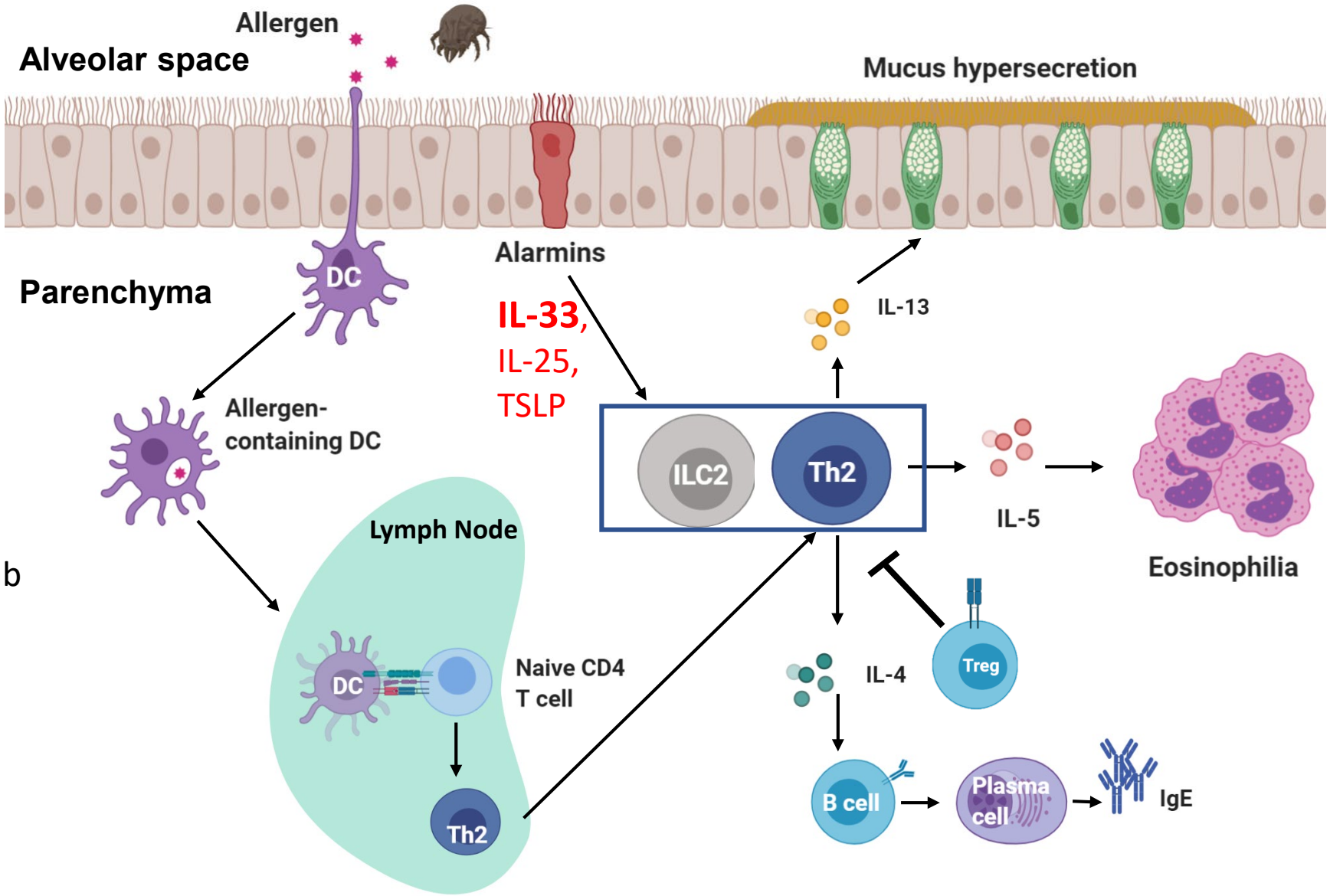
Healthy



Asthmatic

Galli, *et al.* (2008) *Nature* and McCracken, et al. (2017) *JAMA*

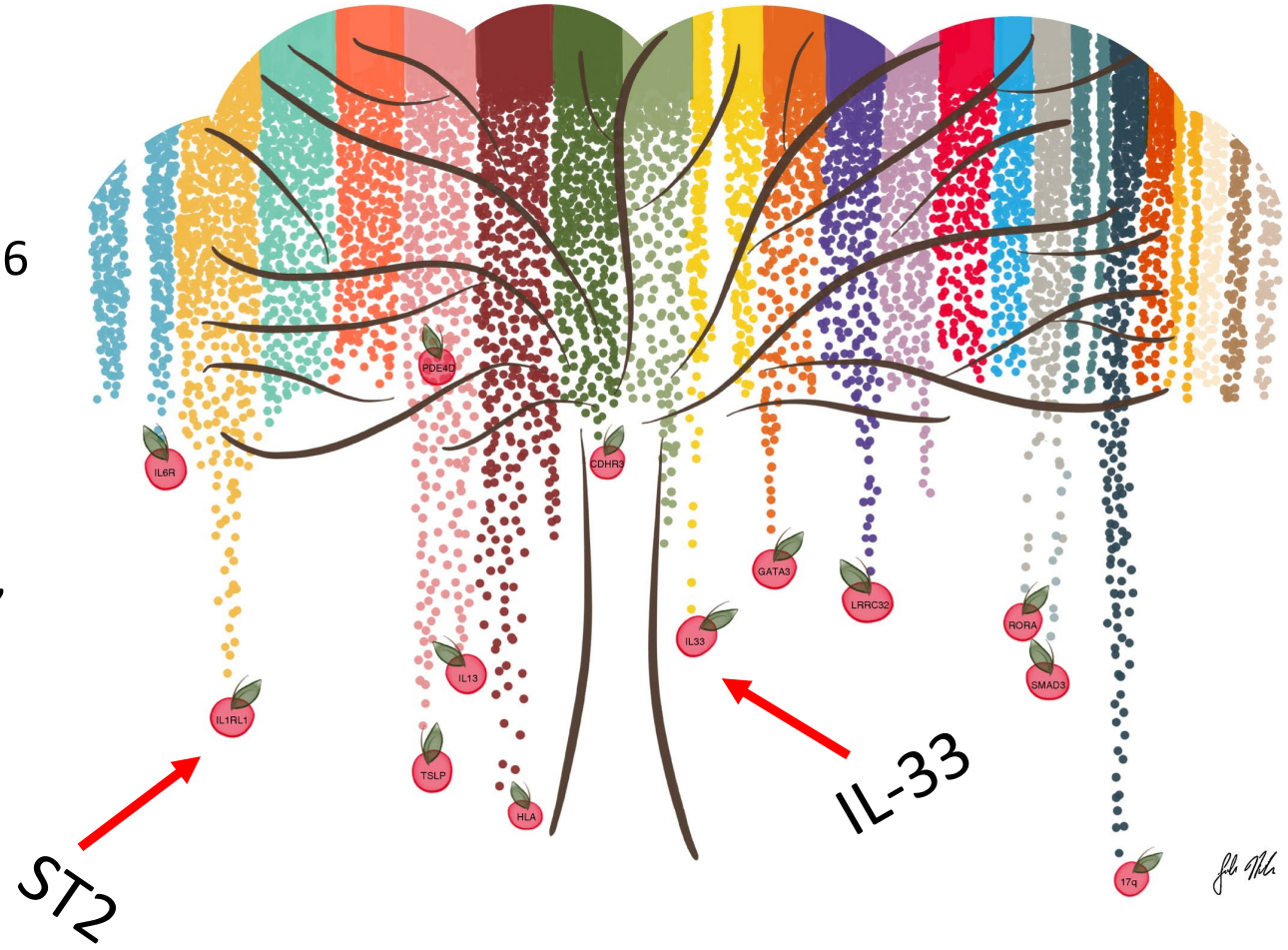
Lung immunity to allergens



Itepekimab

Genome Wide Association Studies (GWAS) to understand the genetic basis of asthma
Low hanging fruit included IL33 and the IL33 receptor (IL1RL1/ST2)

- **EVE Consortium:** diverse ethnic backgrounds in US and Mexico, 2x10^6 SNPs
- **GABRIEL Consortium:** European subjects, 582,892 SNPs, subjects from 23 studies



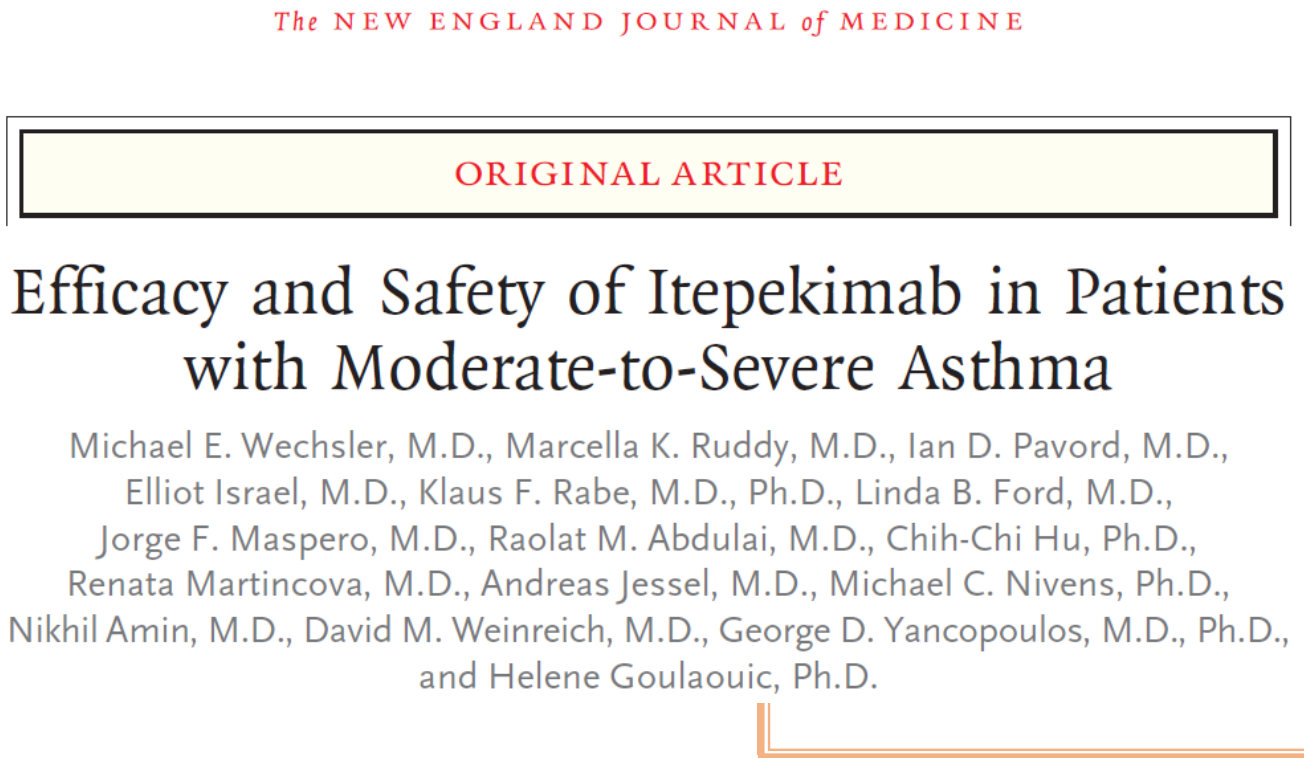
AT THE FOREFRONT
UChicago Medicine

Ober, Carole. *Annals of the American Thoracic Society* (2016): S85-S90.

What is IL-33?

- IL-1 family of cytokines characterized by
  bindin
  recept
  synthe
- Binds t
  - Ca
    re
- Implica
  asthm
  cardio

IL-33

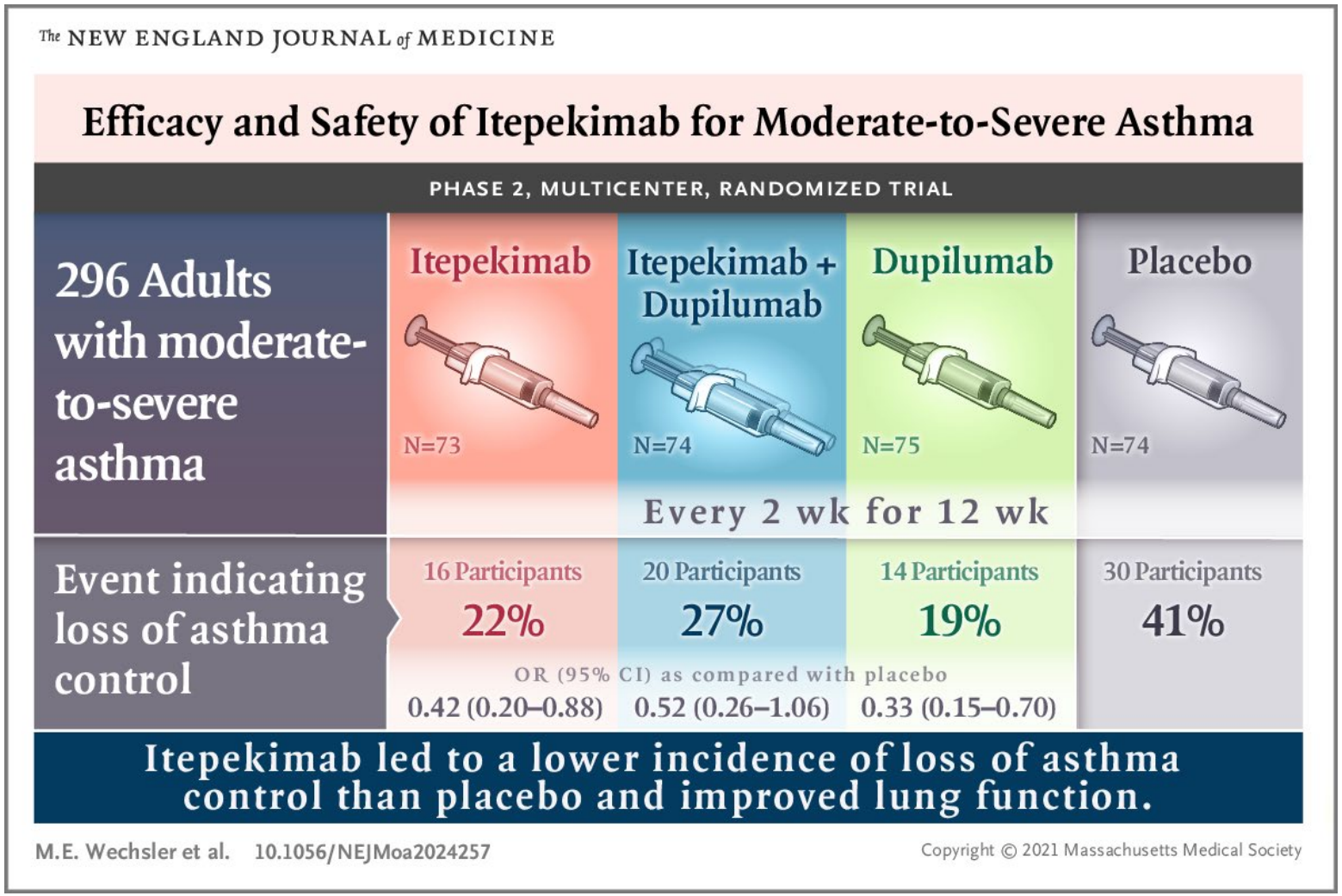


The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Efficacy and Safety of Itepekimab in Patients with Moderate-to-Severe Asthma

Michael E. Wechsler, M.D., Marcella K. Ruddy, M.D., Ian D. Pavord, M.D., Elliot Israel, M.D., Klaus F. Rabe, M.D., Ph.D., Linda B. Ford, M.D., Jorge F. Maspero, M.D., Raolat M. Abdulai, M.D., Chih-Chi Hu, Ph.D., Renata Martincova, M.D., Andreas Jessel, M.D., Michael C. Nivens, Ph.D., Nikhil Amin, M.D., David M. Weinreich, M.D., George D. Yancopoulos, M.D., Ph.D., and Helene Goulaouic, Ph.D.



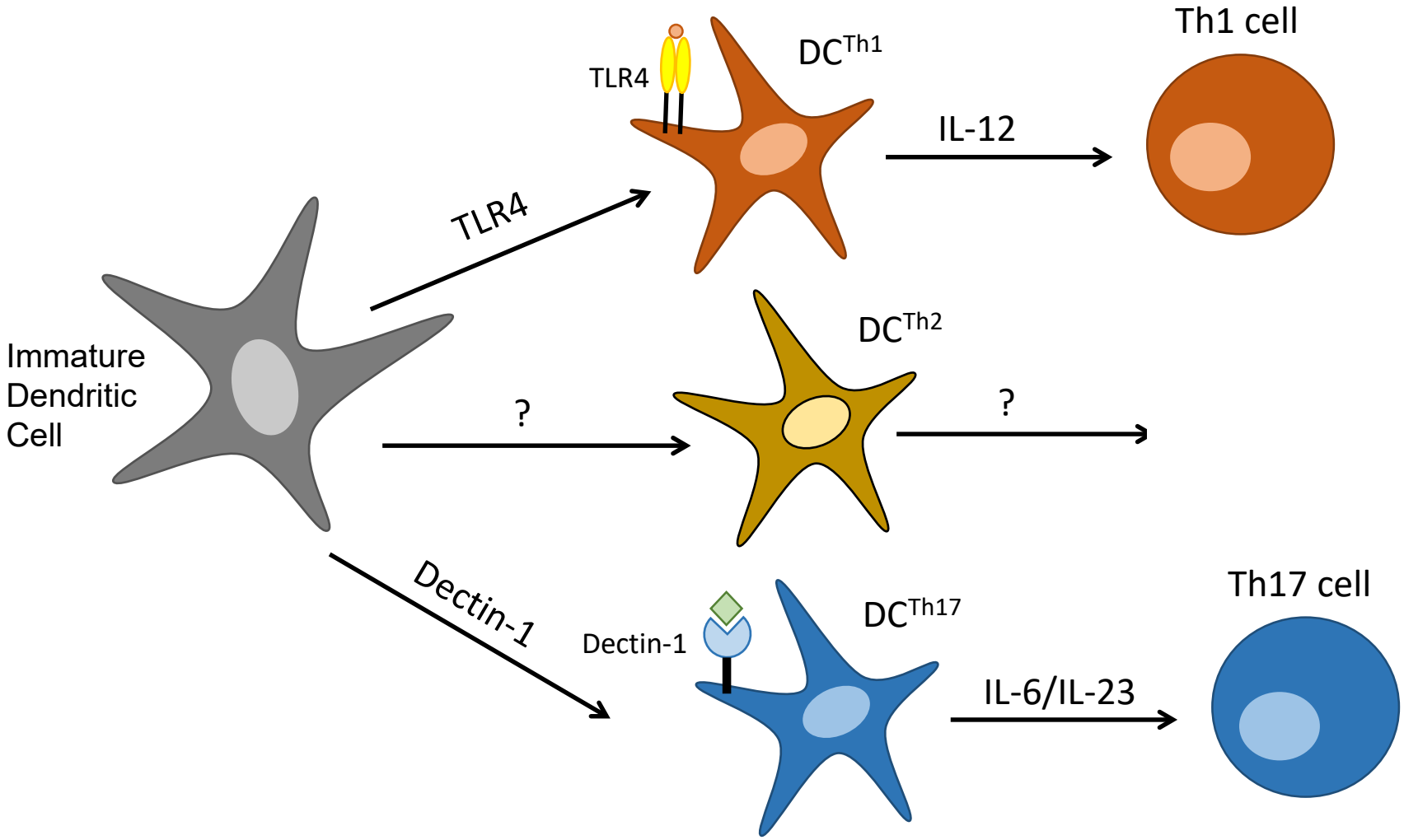
Outline

IL-33 is produced by dendritic cells and is involved in allergic sensitization in the lungs to multiple types of allergens

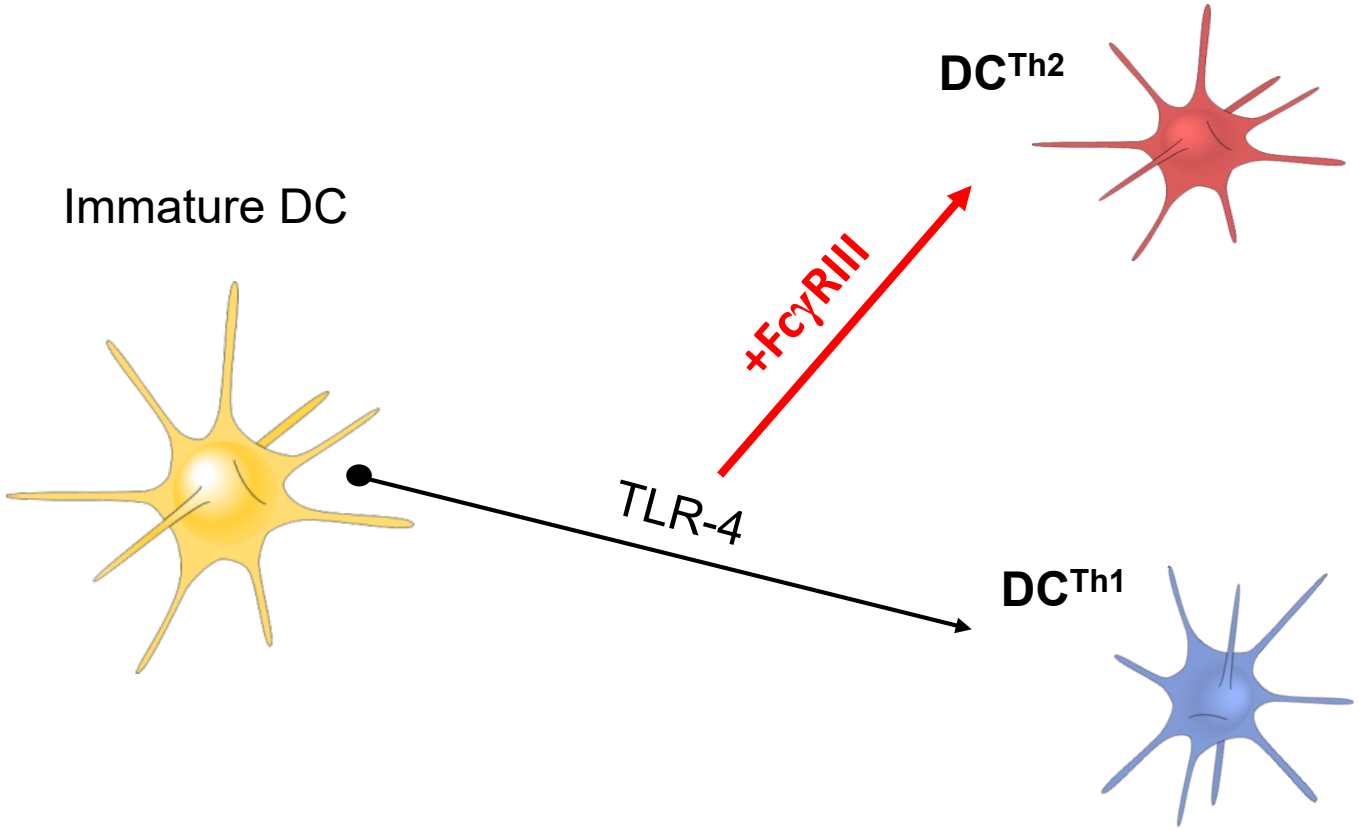
IL-33 levels in Amish farm children are suppressed by their environment,
but Hutterite children IL-33 levels are determined by their genetics

IL-33 is produced by different lung cells in humans compared to mice.

Different stimuli promote development of various T helper responses

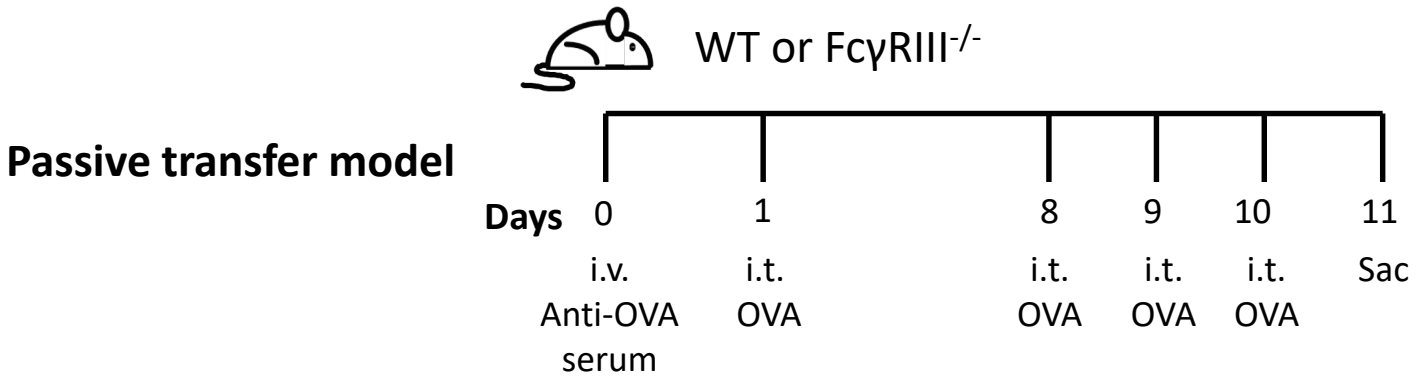


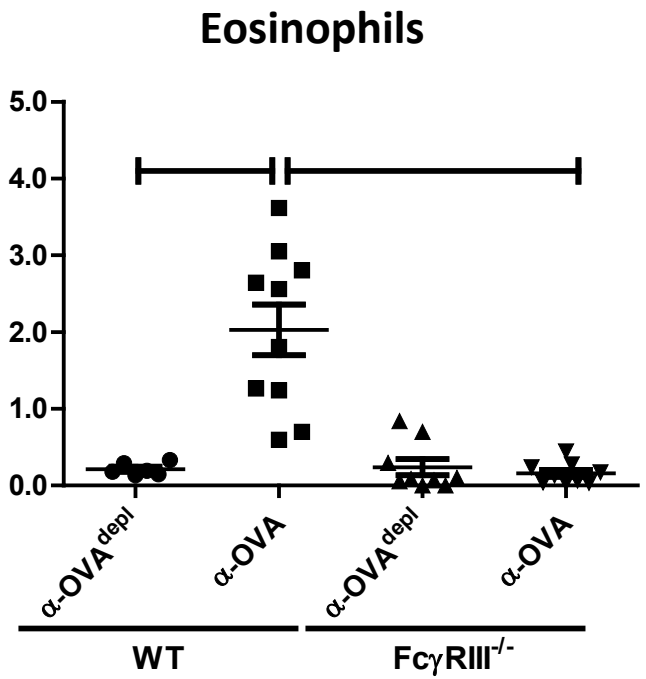
Signaling through FcγRIII diverts TLR-4 stimulated DCs toward DC^{Th2}

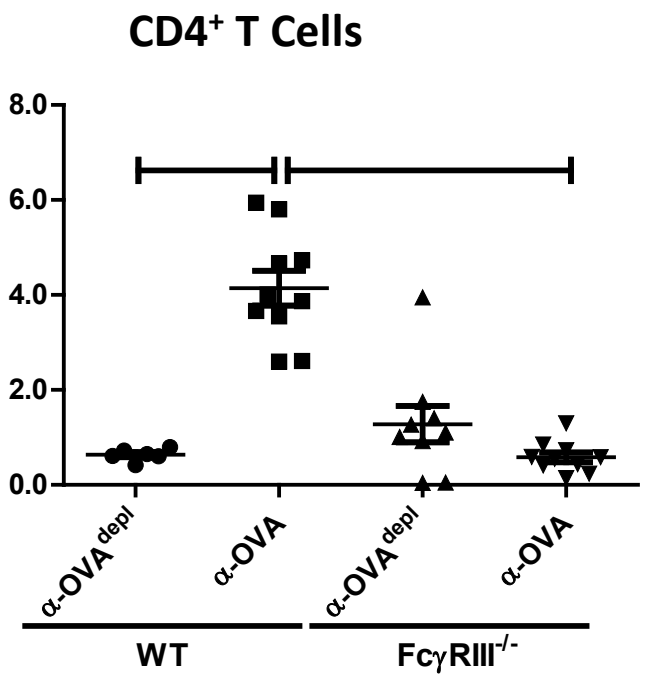


Bandukwala *et al*, (2007) JEM

Passive transfer of antigen specific IgG augments Th2 inflammation upon antigen challenge

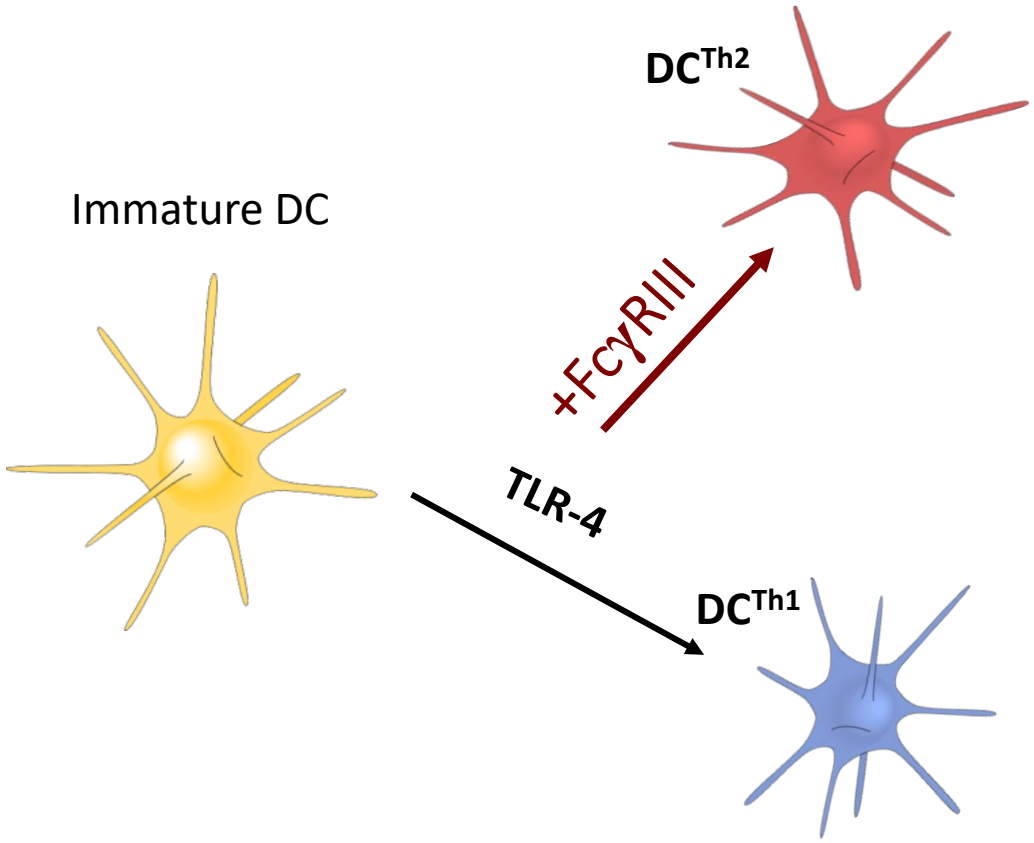


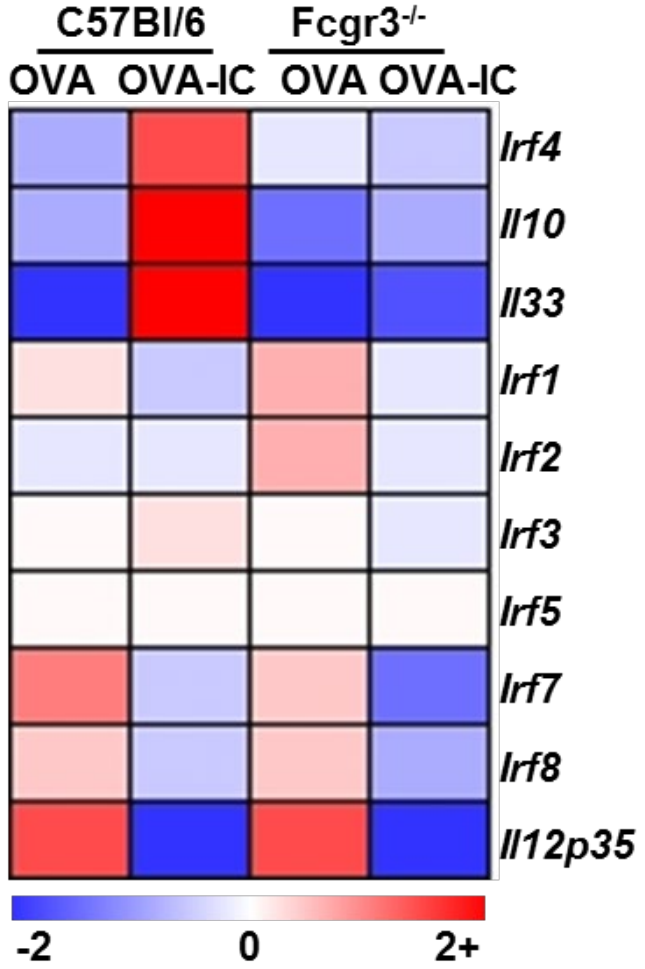




Tjota, et al. JCI. 2013

What genes are regulating DC^{Th2} development?





Bandukwala, et. al. JEM. 2007
Tjota, et, al. JCI. 2013
Williams, et al, Nature Comm 2013

Allergic responses can be triggered by a variety of structurally diverse allergens with varying biological functions

?

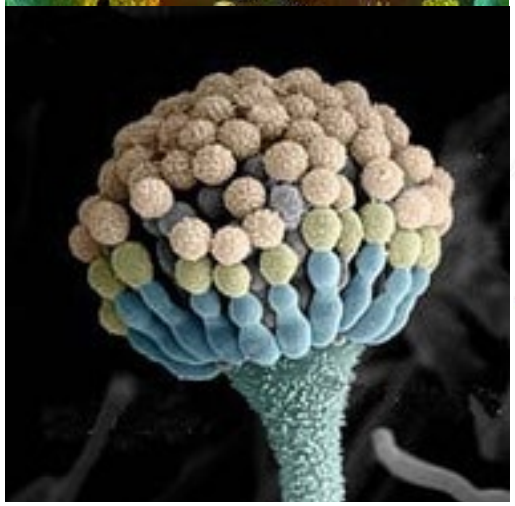
Type I (enzymatic):

- Dust Mite
- Fungal Spores
- Pollen

Type II (non-enzymatic):

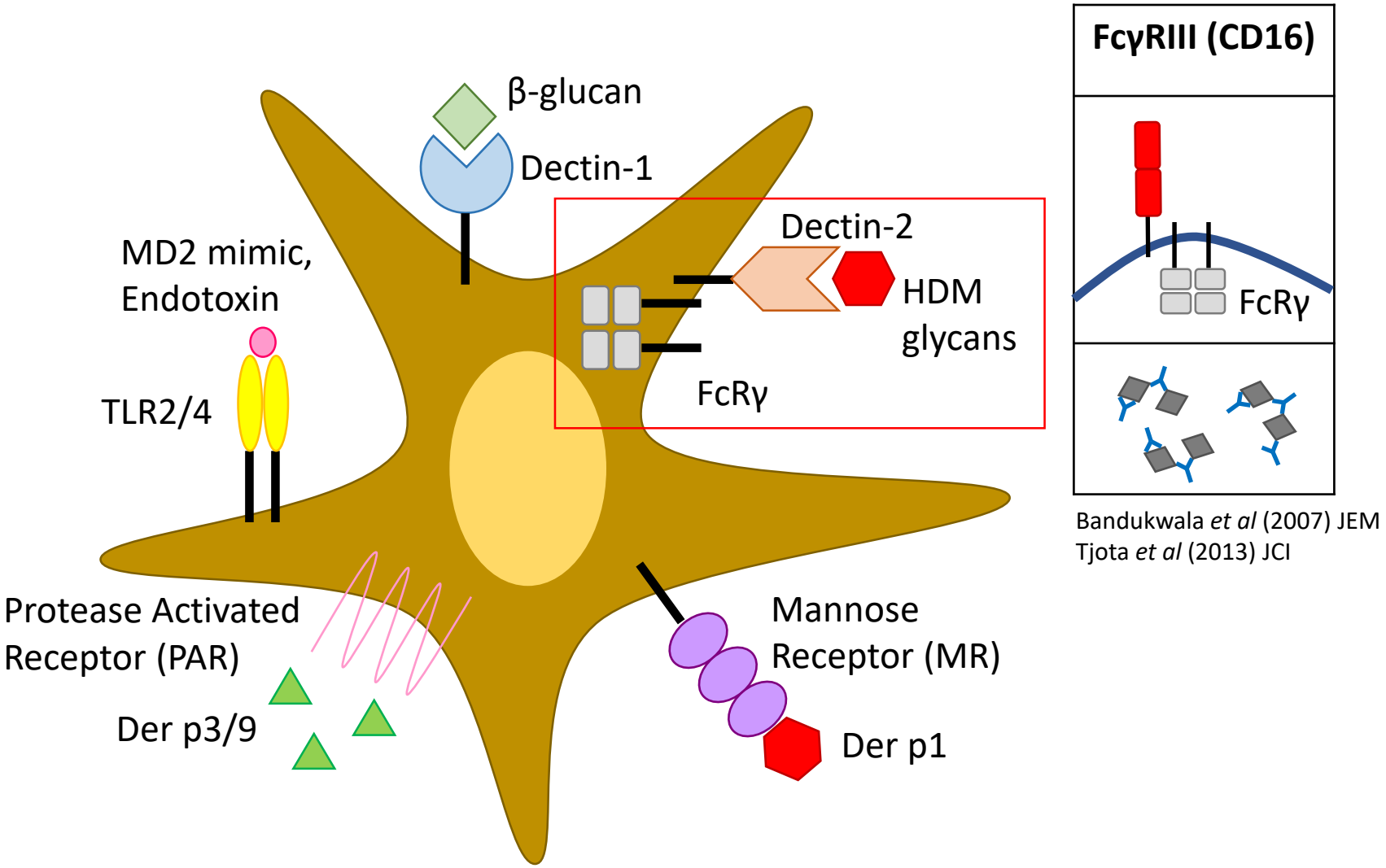
- Animal Dander
- Latex
- Ovalbumin
- Wheat Flour

Immune complexes



Erwin and Platts-Mills (2005) Immunol Allergy Clin North Am

HDM extract targets multiple cellular pathways

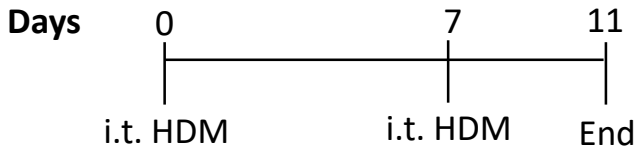


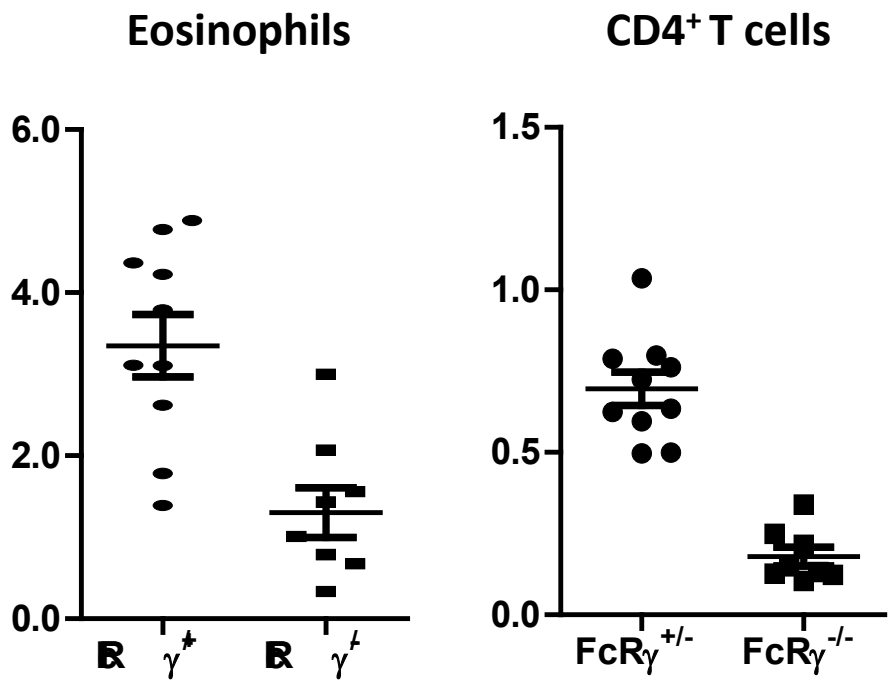
Bandukwala *et al* (2007) JEM
Tjota *et al* (2013) JCI

Is HDM-mediated Th2 inflammation FcRγ dependent?

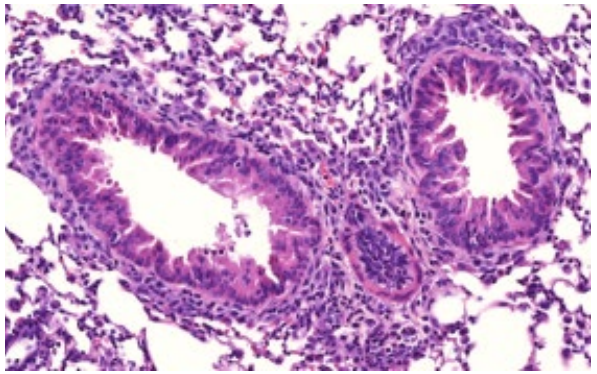
HDM-mediated allergic airway inflammation is FcRγ-dependent

FcRγ$^{+/-}$ and FcRγ$^{-/-}$

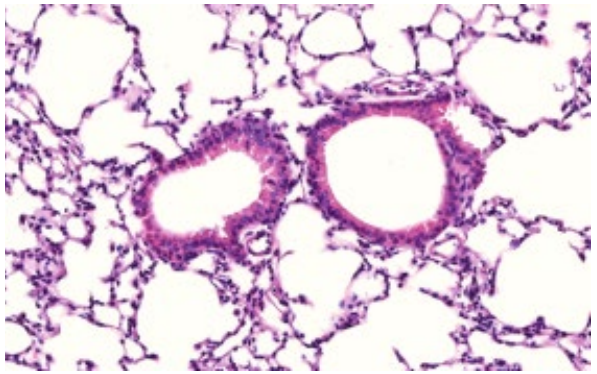




FcRγ$^{+/-}$



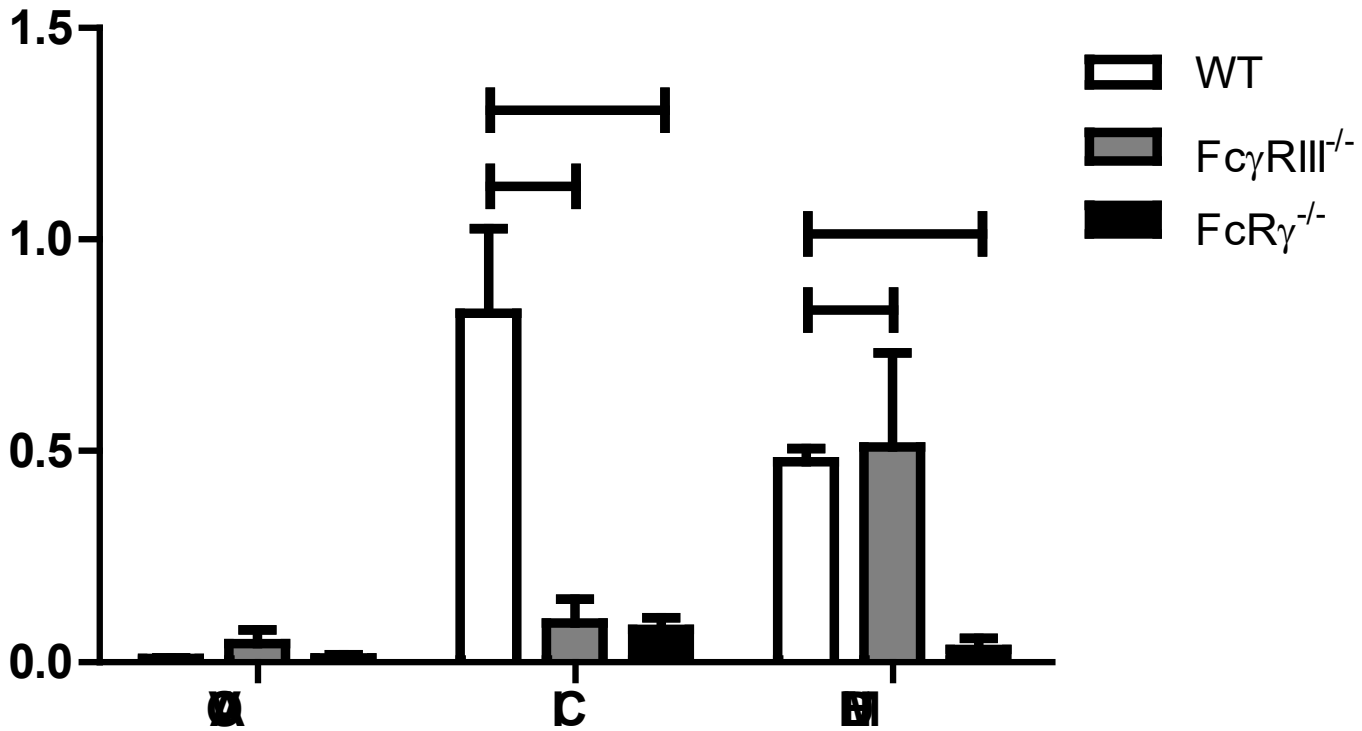
FcRγ$^{-/-}$



Tjota et al, J. Allergy Clin Immun. 2014

HDM upregulates IL-33 in BMDCs in an FcRγ-dependent manner

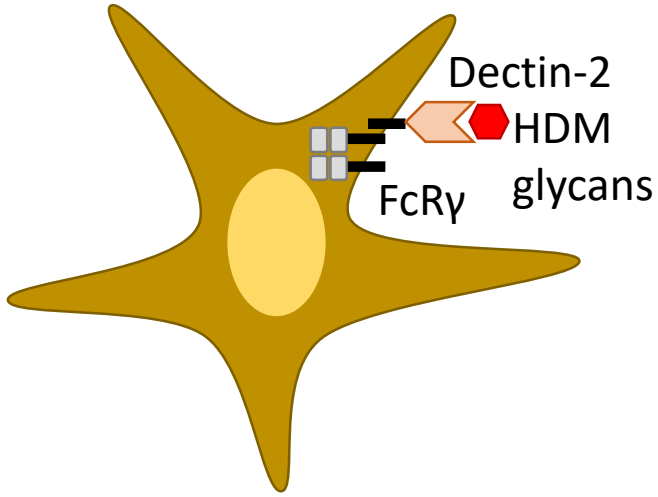
Generated BMDCs → Treated the cells overnight with OVA, IC, or HDM → Isolated the RNA for qPCR

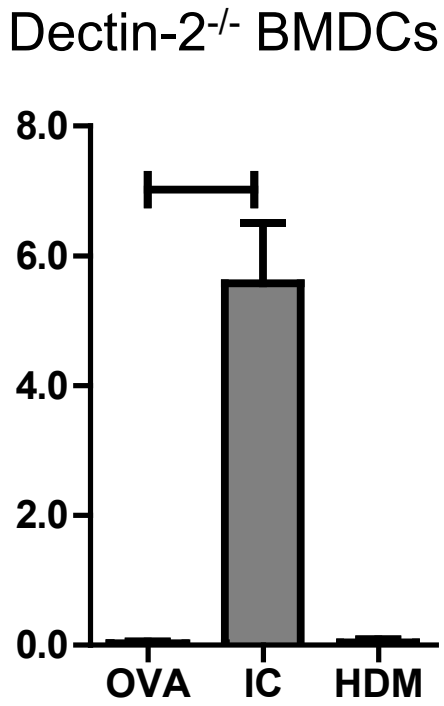


Tjota et al, J. Allergy Clin Immun. 2014

HDM-induced IL-33 upregulation in BMDCs is Dectin-2 dependent

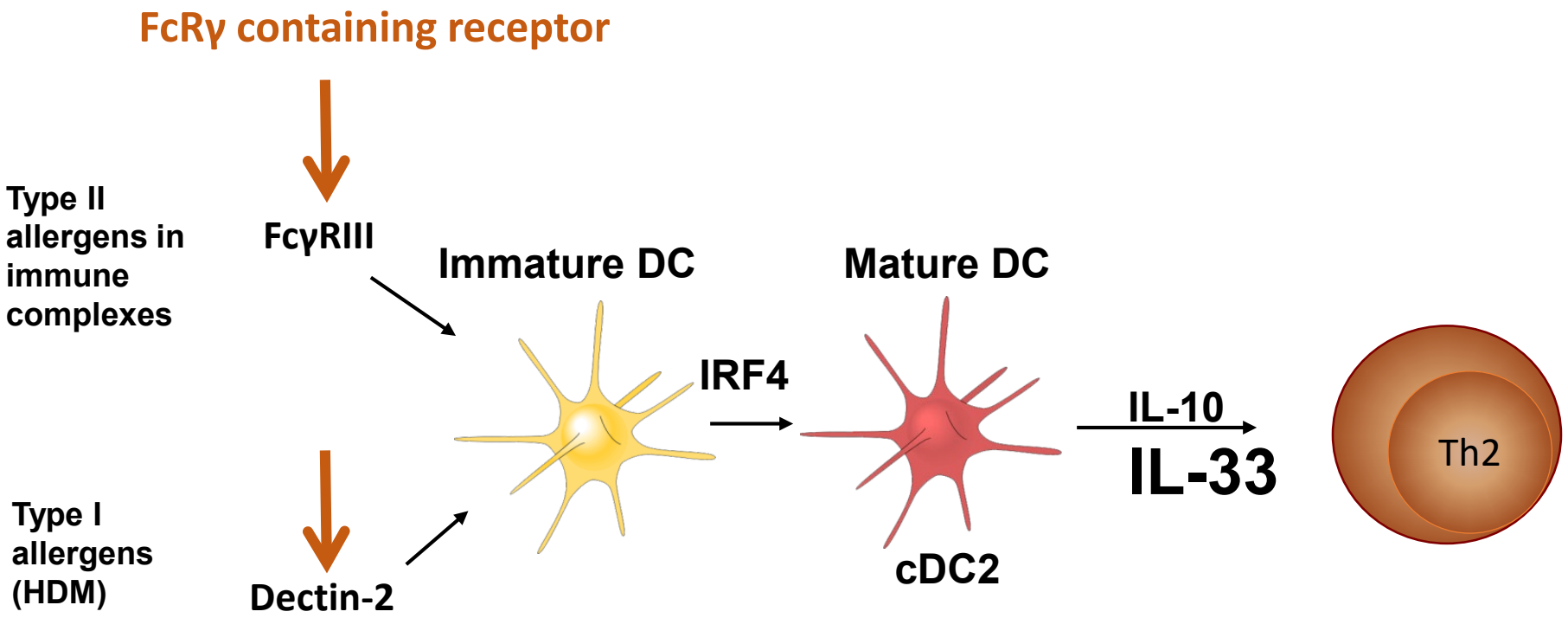
Generated Dectin-2$^{-/-}$ BMDCs → Treated the cells overnight with OVA, IC, or HDM → Isolated the RNA for qPCR





Dectin 2$^{-/-}$ bones provided by N. Barrett

Stimulation of FcRγ-containing receptors can lead to increased DC expression of IRF4 resulting in DC^{Th2} skewing



Bandukwala et al J. Exp Med. 2007
Tjota et al, J. Clin. Invest. 2013
Williams et al, Nature Comm 2013
Tjota et al, J. Allergy Clin Immun. 2014
Camacho, et al. JCI Insight, 2022

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IL-33 is produced by different lung cells in humans compared to mice.

The Farm Effect: Early childhood exposure protects against asthma and allergies

Erika von Mutius, JACI 2004

Amish farm children have a low asthma and atopy risk compared to Hutterites farm children

Previous Studies	Asthma	Allergic Sensitization (skin prick)
Amish children 6-12 (Holbreich *et al*. 2012)	5.2%	7.2%
Hutterite children 6-10 (Motika *et al*. 2011)	21.3%	33.3%

Amish

mage credit: http://static.guim.co.uk/sys-images/Guardian/Pix/pictures/2012/8/1/1343834102806/Amish-A-Secret-Life-008.jpg

- Anabaptist founder populations from Europe
- Large families
- Long nursing time
- Germanic farming diet
- Consume raw milk
- Vaccinated
- Cats and dogs not allowed in home
- No television/radio exposure
- Children spend a lot of time outdoors
- No obesity in children
- ***High level of genetic similarity between the two founder populations (by SNP analysis)***

Hutterite

Farm environment and exposures differ between Amish and Hutterite communities

Current Opinion in Immunology

Amish

- Family farms
- Traditional farming techniques
- Women and children help farm

Hutterites

- Communal farms
- Farming highly mechanized
- Women and children not involved in farming

Hypothesis

Amish children live in an environment that reduces risk of allergic sensitization through modulation of the immune response

Methods:

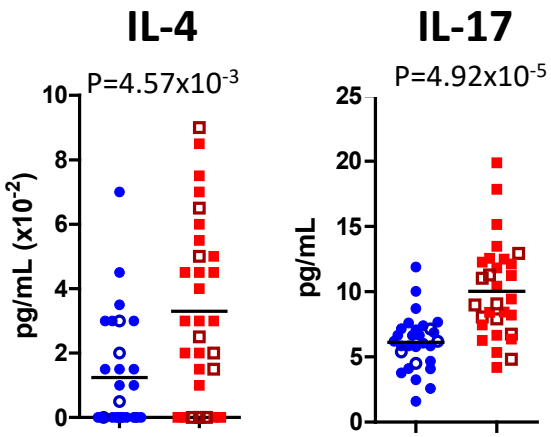
- Collect blood from 30 Amish and 30 Hutterite children
- Determine the specific changes in **peripheral blood leukocyte (PBL)** gene expression, cell phenotypes, and functions that could lead to reduced allergic sensitization.
- Tested the effect of house dust in a mouse model of asthma

Study Sample

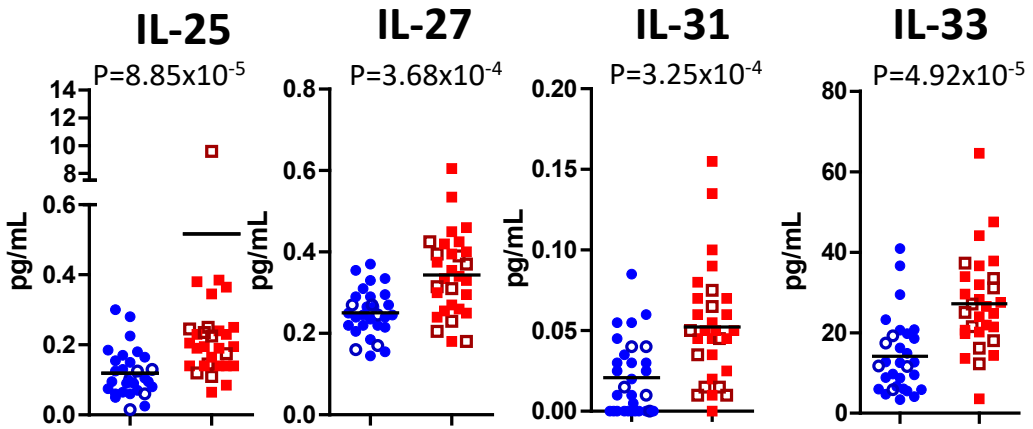
Stein, Hrusch, Gozdz, et al. NEJM 2016

PBLs from Amish children produce less cytokine in response to LPS

- Non-atopic Amish
- Atopic Amish
- Non-atopic Hutterite
- Atopic Hutterite

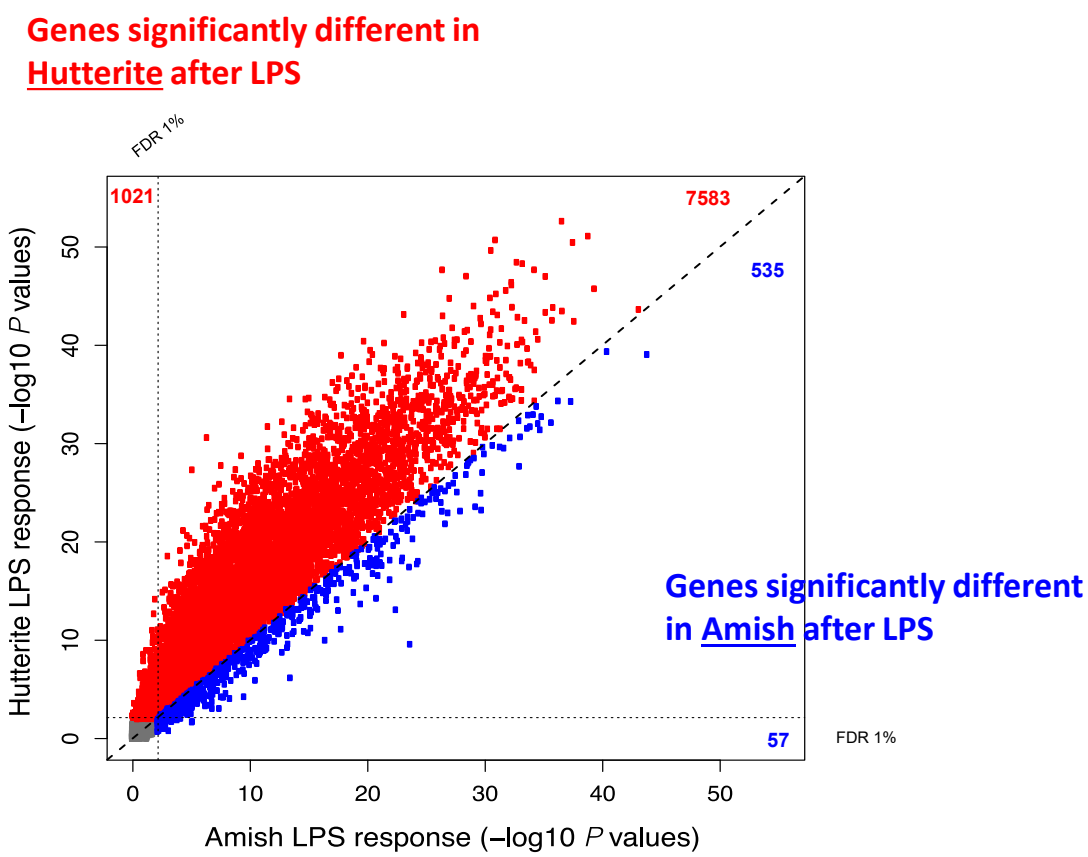


IL-2, IL-5, IL-15, and IL-22 were trending down in the Amish



- No difference observed in **IL-10**, IL-9, IL-13, **IL-12p70**, GM-CSF, MIP3A, IL-1β, IL-6, TNFα, IL-28A, IL-23, or IFNγ

Amish PBL have a globally dampened transcriptional response to LPS:



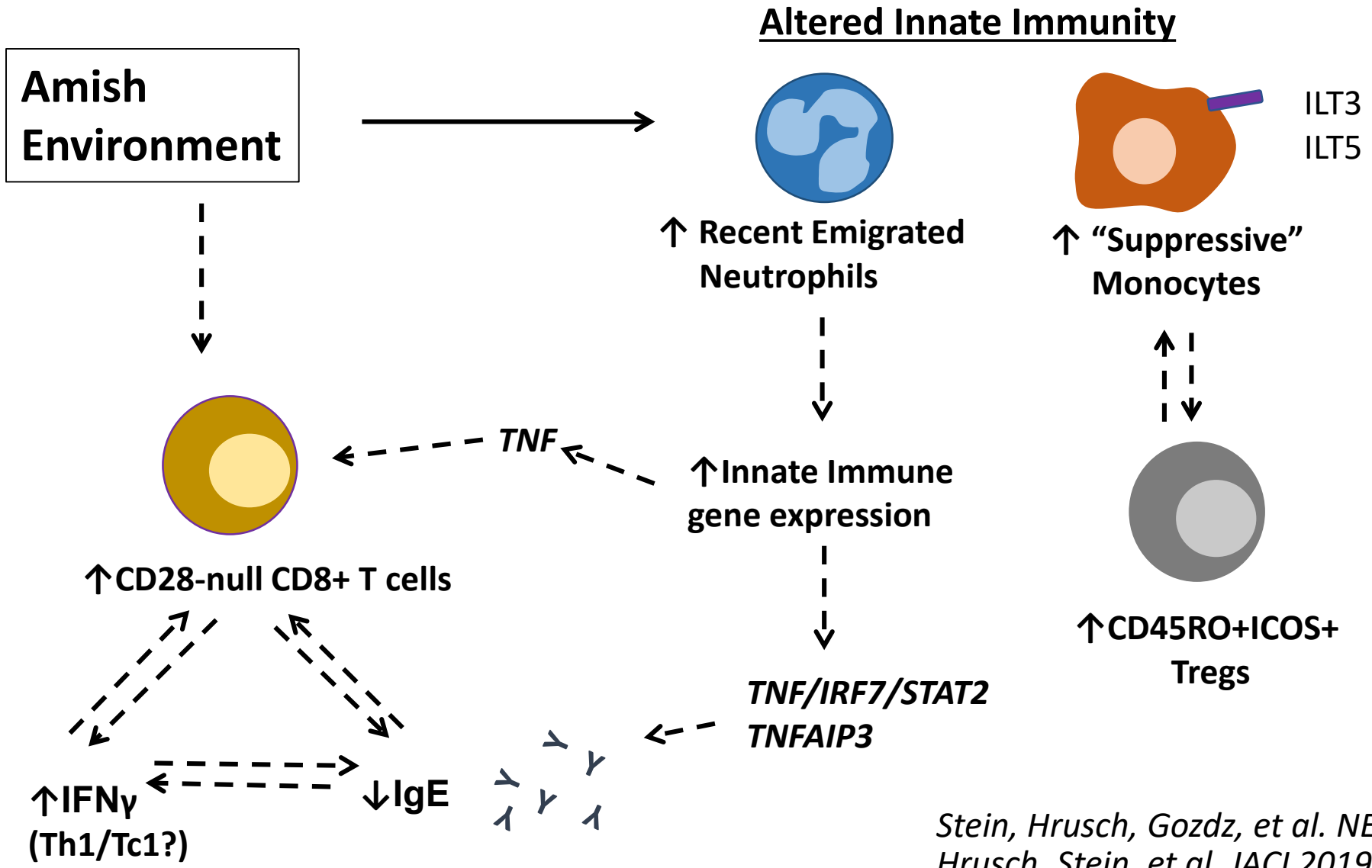
Conclusions:

Amish kid's blood cells have a higher basal level of neutrophils and gene transcription of innate genes in neutrophils, but are hypo-responsive to innate stimulation.

Evidence of hyper-reactivity in a "bored" immune response in Hutterite children?

Tregs may be directly involved in the suppression of the innate immune cells in the Amish children.

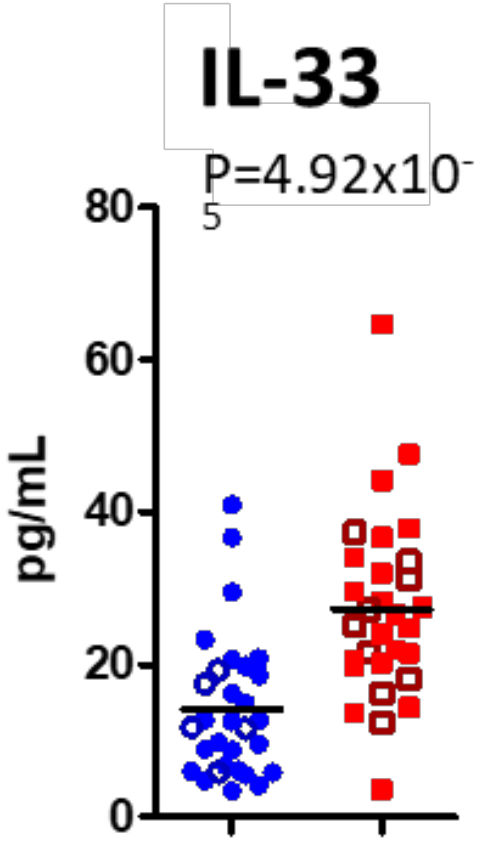
Conclusions:
Effects of Amish environment on circulating immune cells:
Potential mechanisms for reduced asthma risk

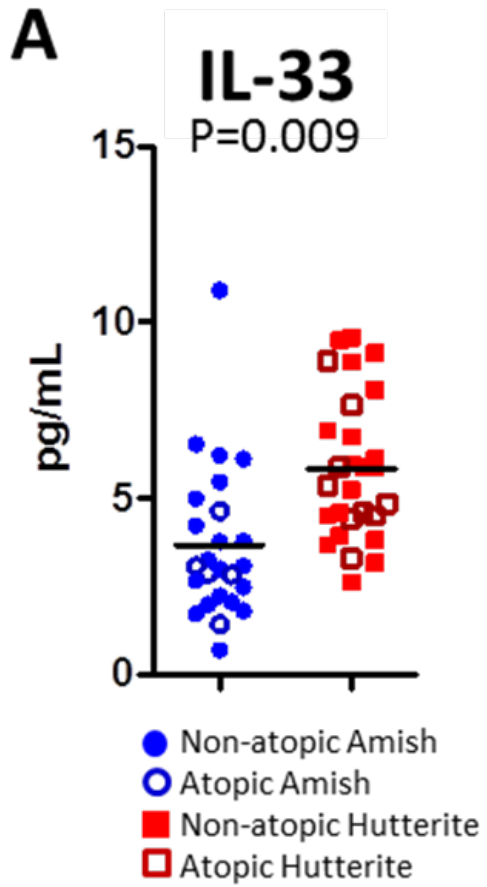


Stein, Hrusch, Gozdz, et al. NEJM 2016
Hrusch, Stein, et al. JACI 2019

PBLs from Amish children produce less IL-33 cytokine in response to LPS and at baseline

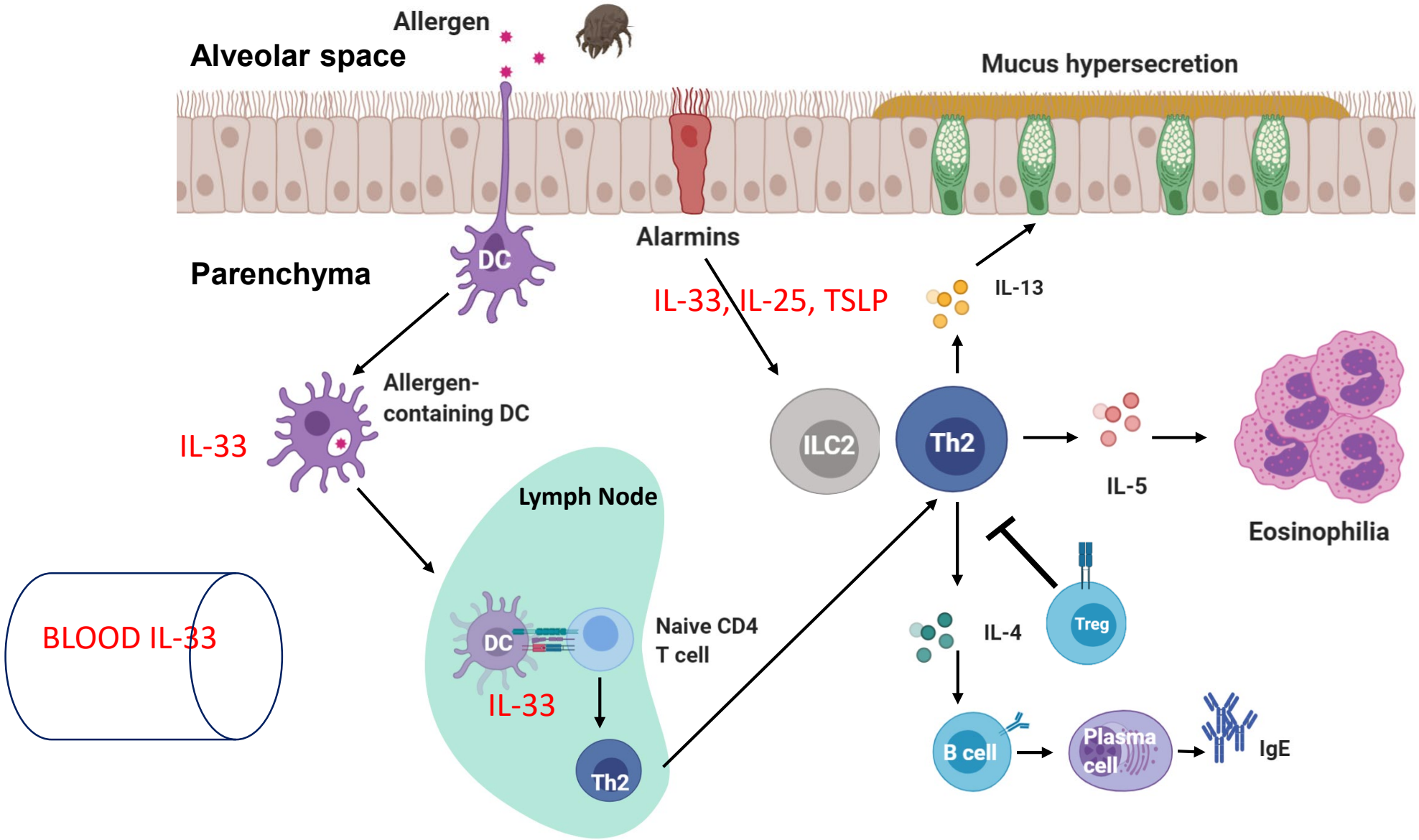
- Non-atopic Amish
- Atopic Amish
- Non-atopic Hutterite
- Atopic Hutterite





At baseline, IL-33 was the only cytokine that was different between the Amish and Hutterite children

Lung immunity to allergens



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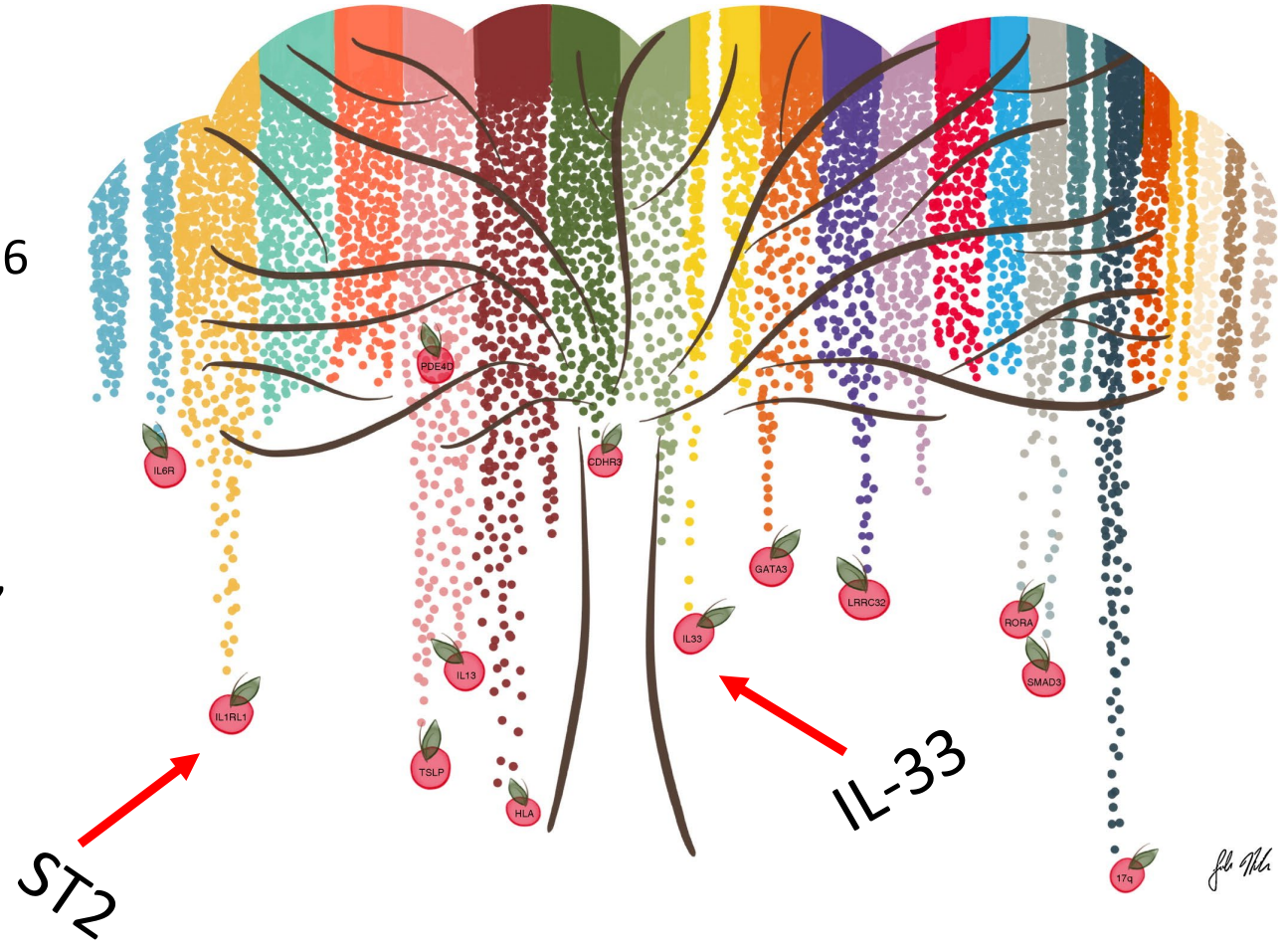

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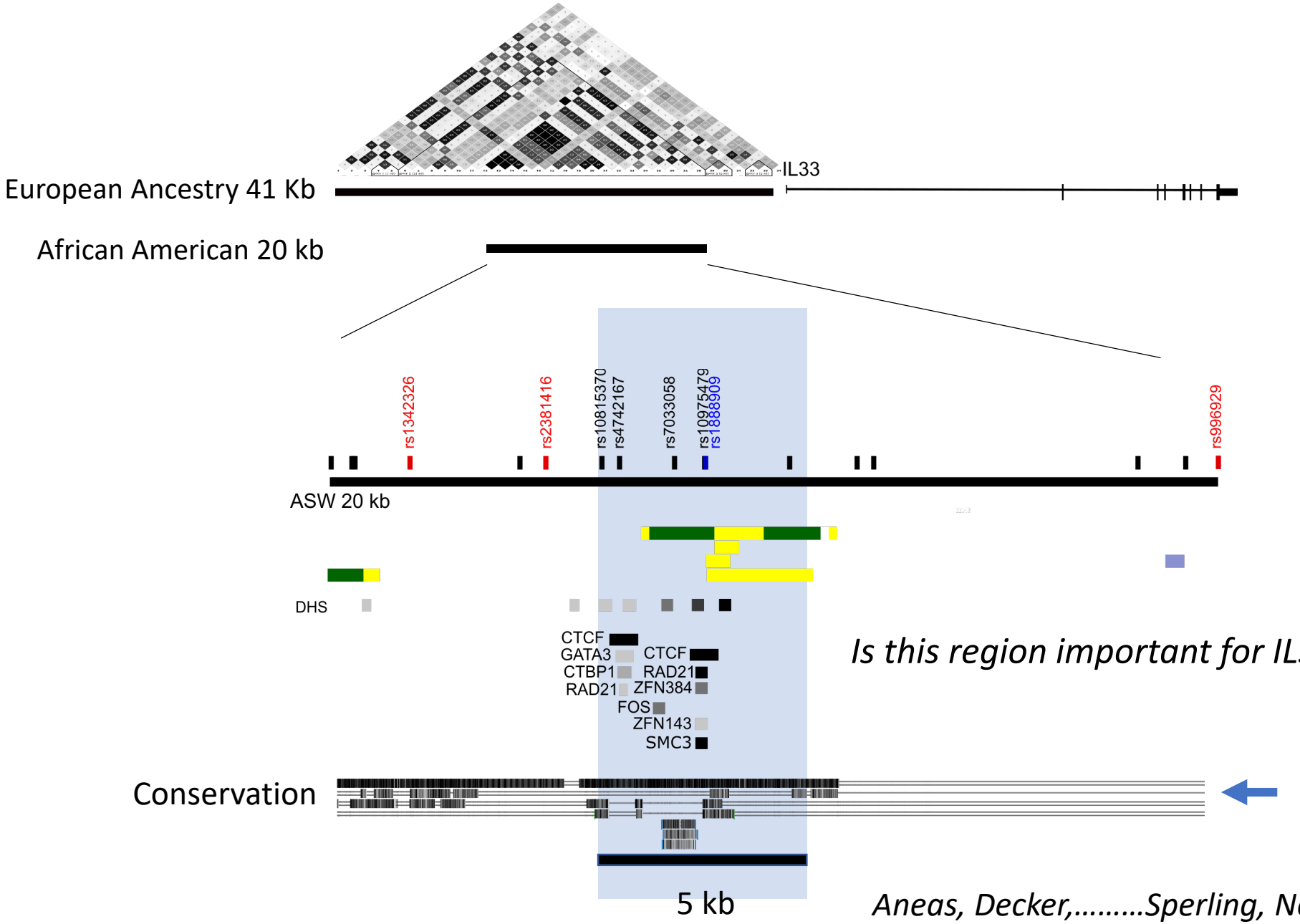
UChicago Medicine

Ober, Carole. *Annals of the American Thoracic Society* (2016): S85-S90.

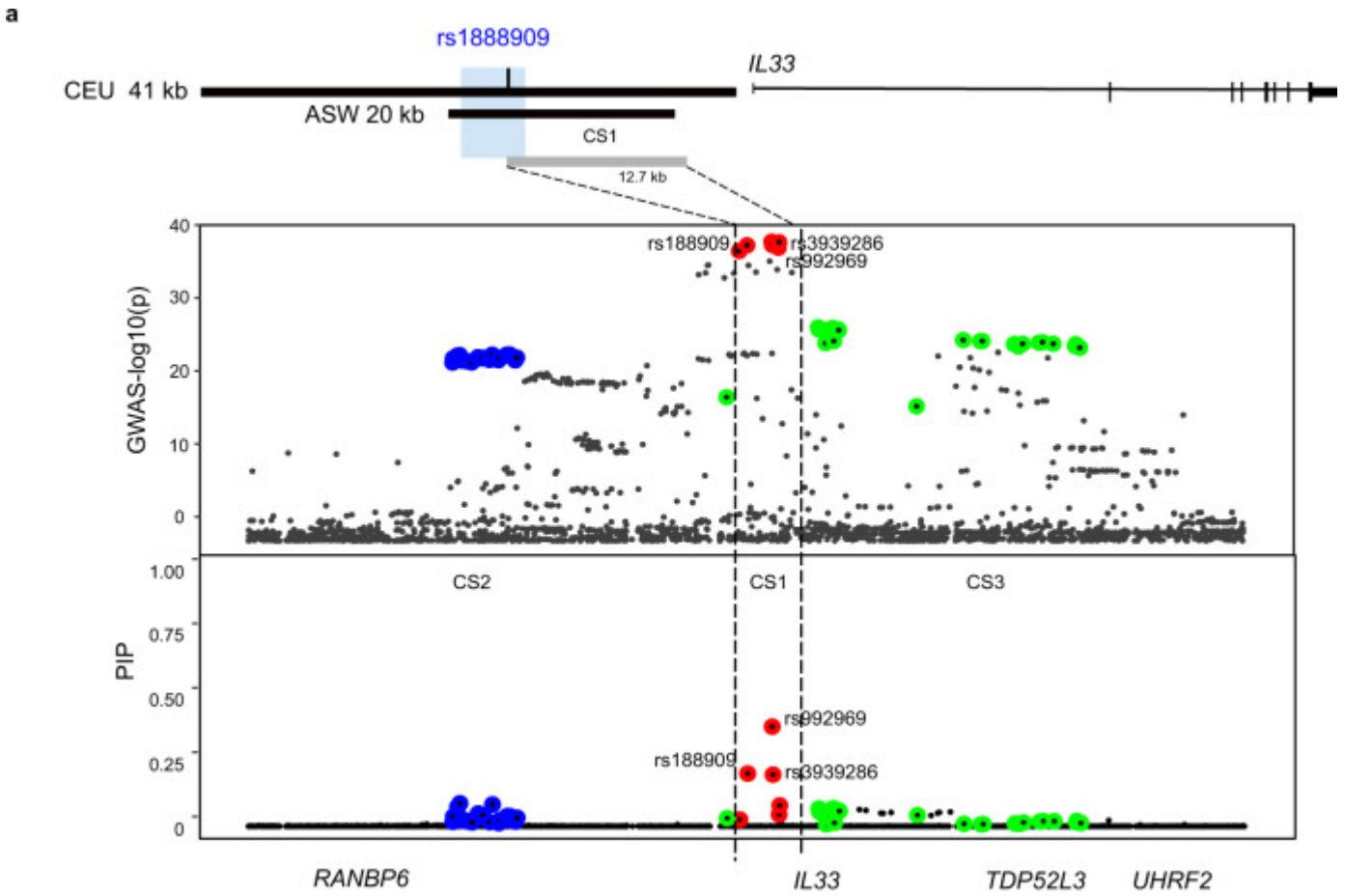
SNPs associated to increased asthma risk are located upstream *of the IL33* gene



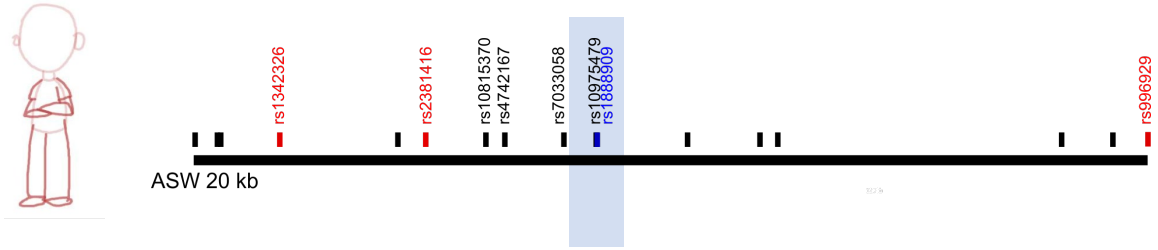
Is this region important for IL33 expression in vivo?

Aneas, Decker,……Sperling, Nobrega. Nat Comm 2021

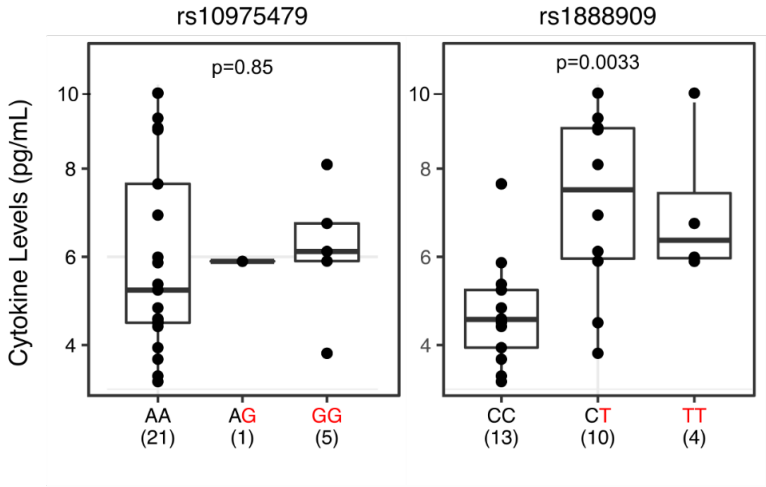
Bayesian fine-mapping quantifies uncertainty in variable selection



Risk allele of GWAS SNP rs1888909 is associated with *IL33* mRNA and IL-33 protein levels



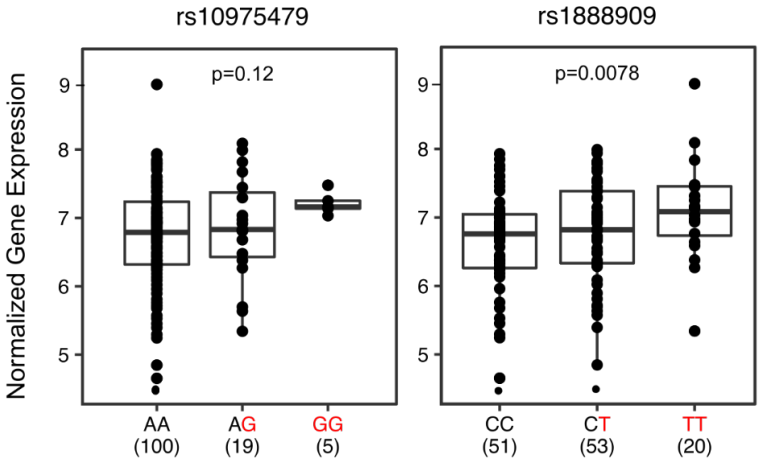
Protein levels (plasma) **From Hutterite Children**



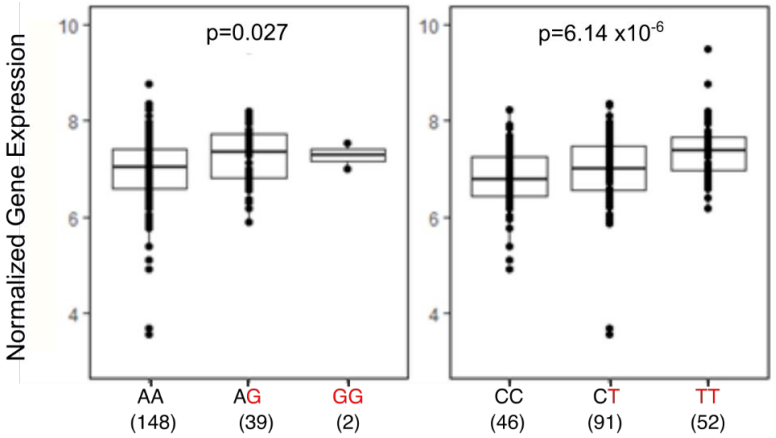
No association was found in Amish children's plasma suggesting that the farming environment trumped their genetics

AEC mRNA

Endobronchial Brushings



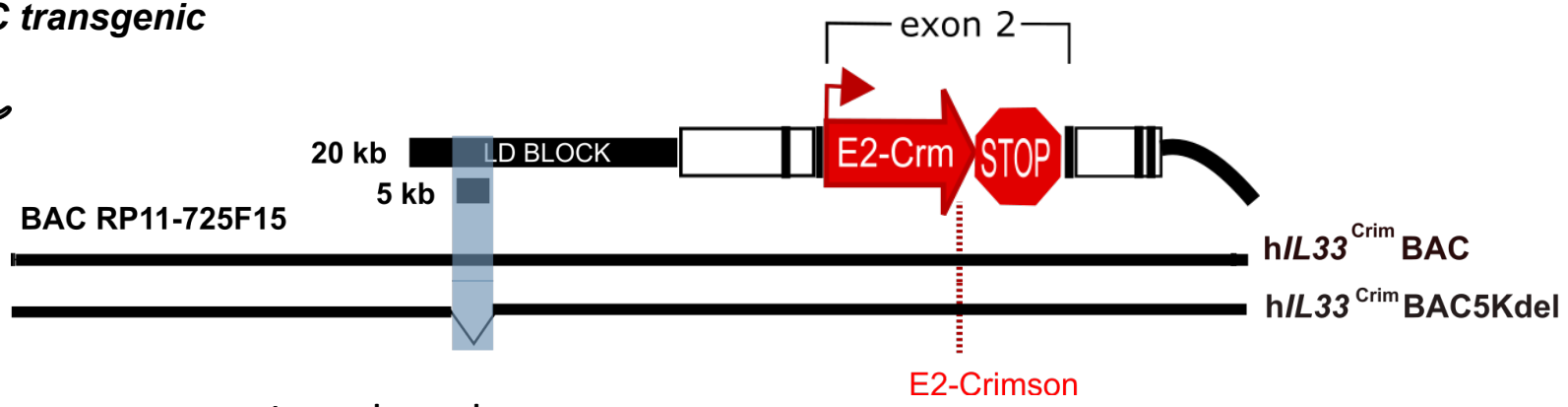
Nasal Epithelial Cells



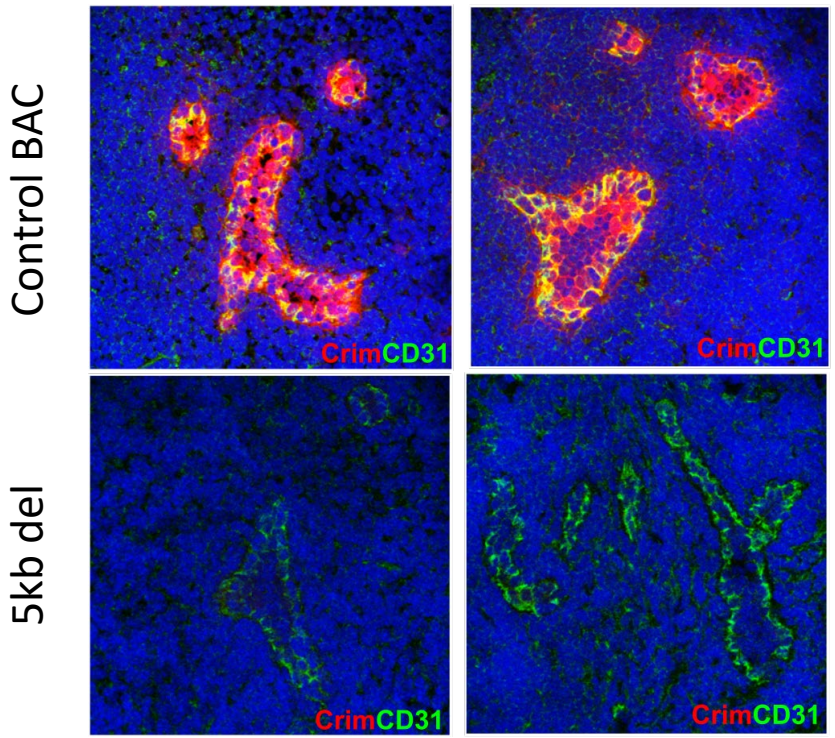
Aneas, I. Decker, D. …….Sperling, AI, Nobrega, M. Nature Comm 2021

The 5 kb asthma-associated region contain important regulatory activity

" Humanized" mouse BAC transgenic



Lymph node

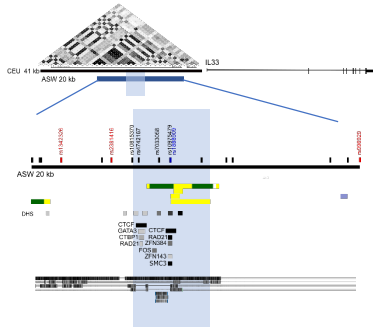


What is the specific regulatory function of this region?

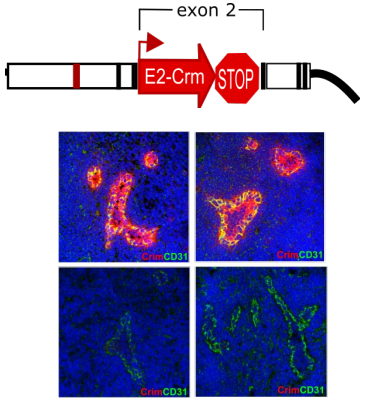
Aneas, Decker,……Sperling, Nobrega. Nat Comm 2021

IL-33 is regulated by a causal SNP

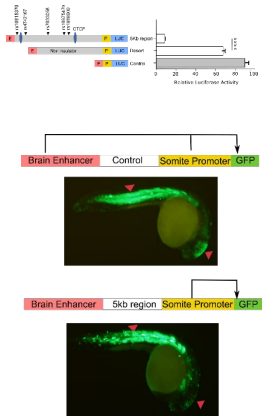
LD block upstream *IL33*



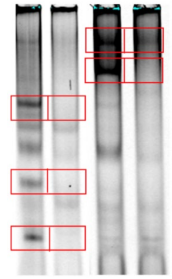
5 kb regulatory region



↓ Enhancer blocking Activity of risk haplotype

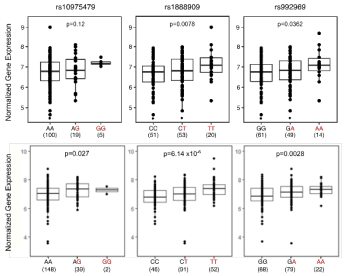


Differential protein complexes risk vs non-risk



TF candidate identification OCT1

Rs1888909-T ↑ IL33 expression



↑ Asthma risk

Aneas, Decker,……Sperling, Nobrega. Nat Comm 2021

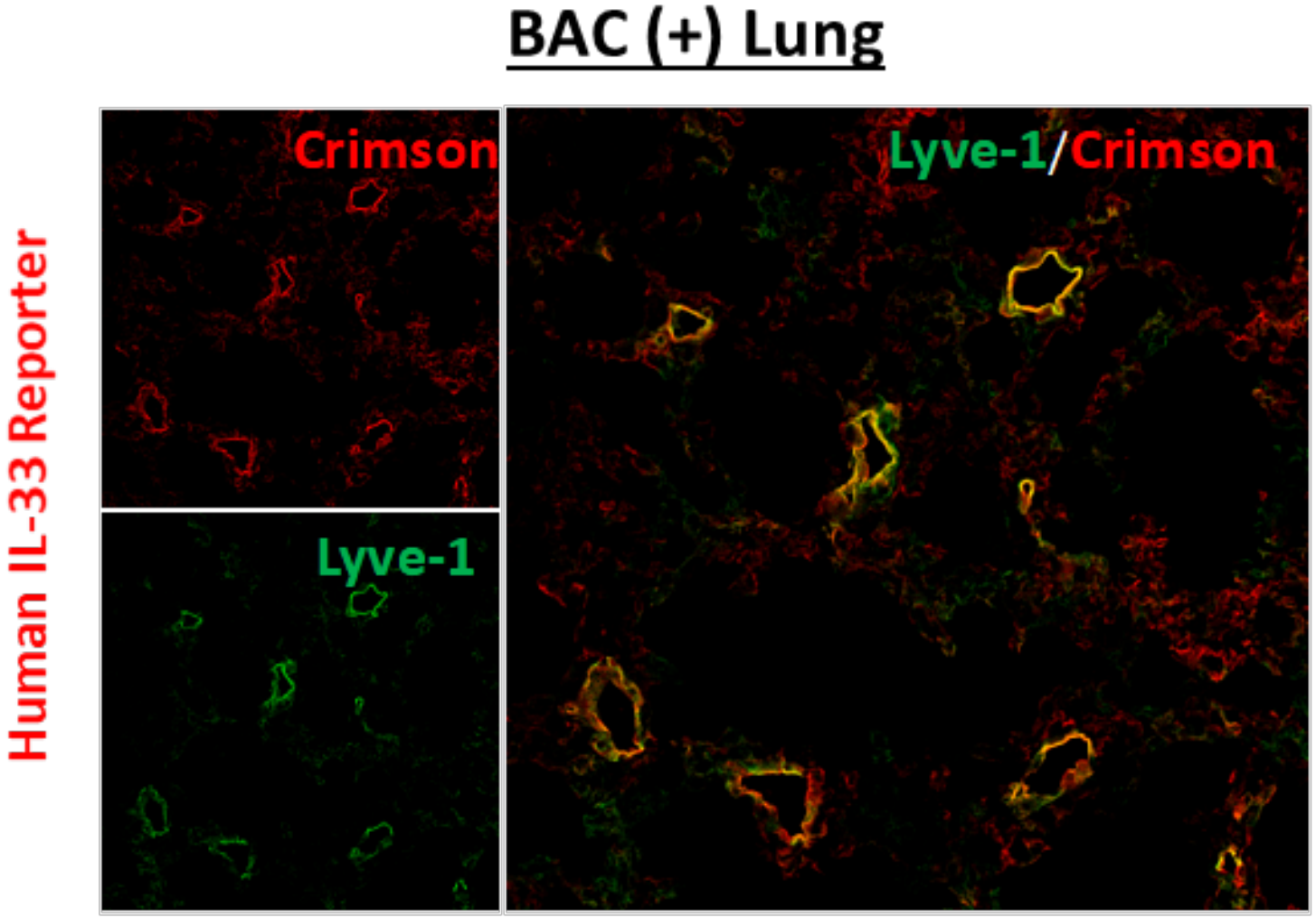
Outline

IL-33 is produced by dendritic cells and is involved in allergic sensitization in the lungs to multiple types of allergens

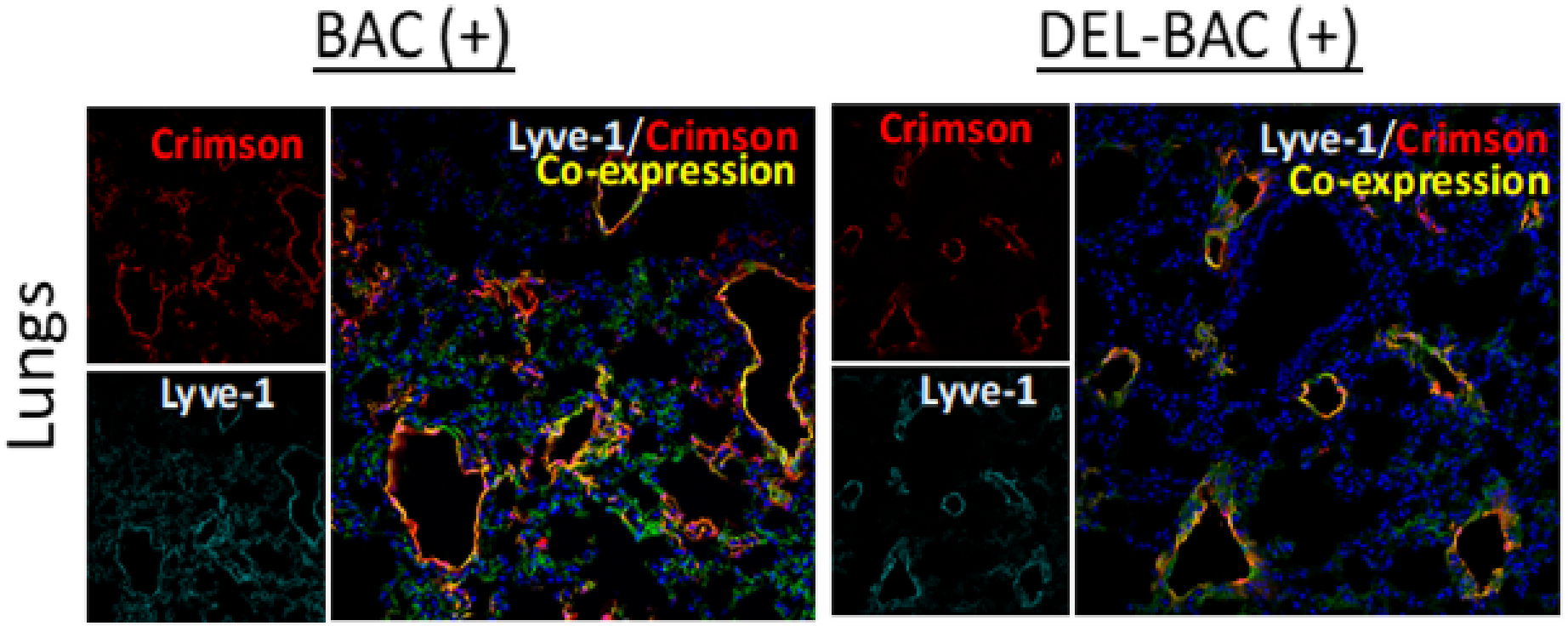
IL-33 levels in Amish farm children are suppressed by their environment,
but Hutterite children IL-33 levels are determined by their genetics

IL-33 is produced by different lung cells in humans compared to mice.

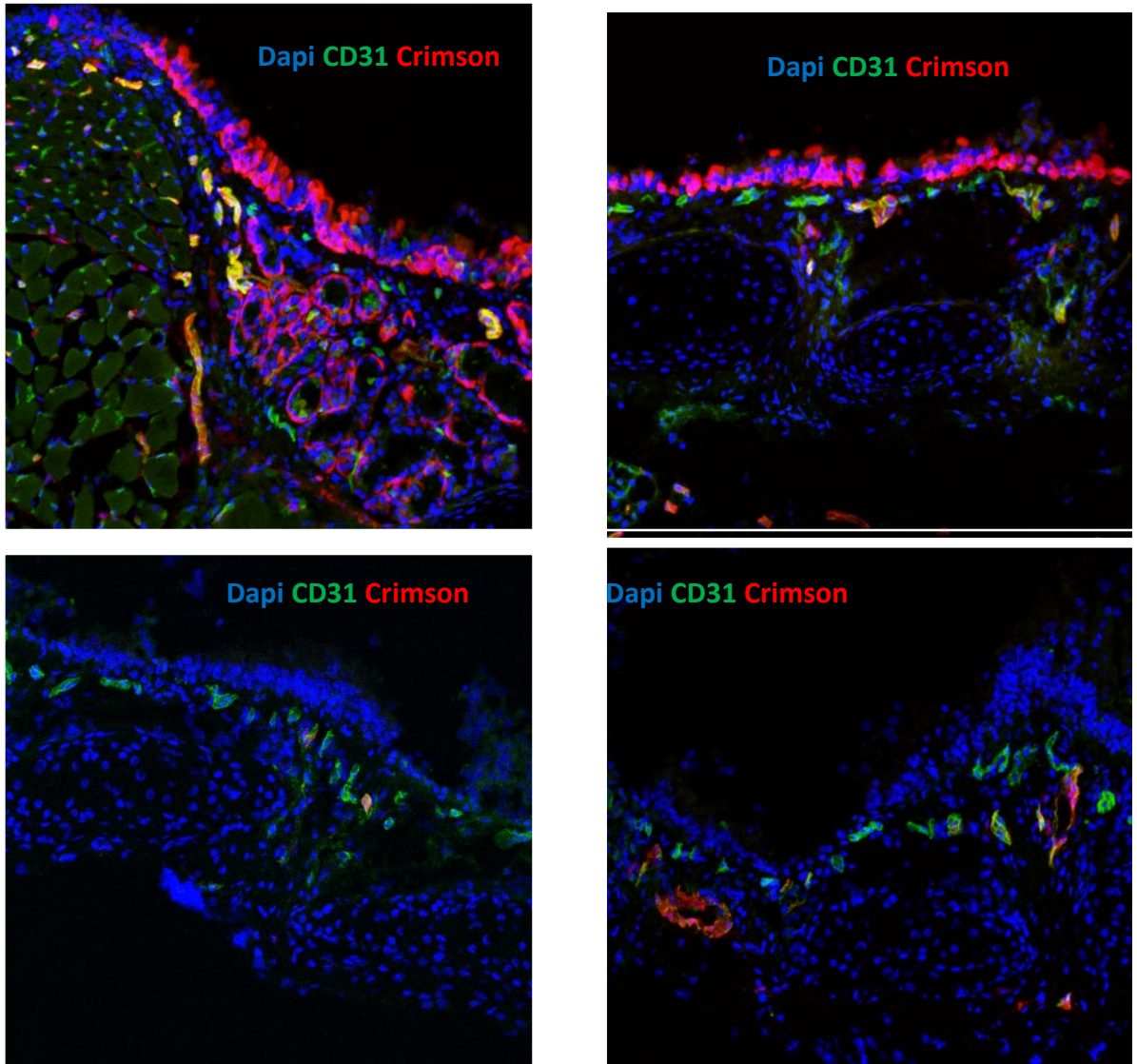
In the lungs, human *IL33*-reporter is expressed primarily in endothelial cells



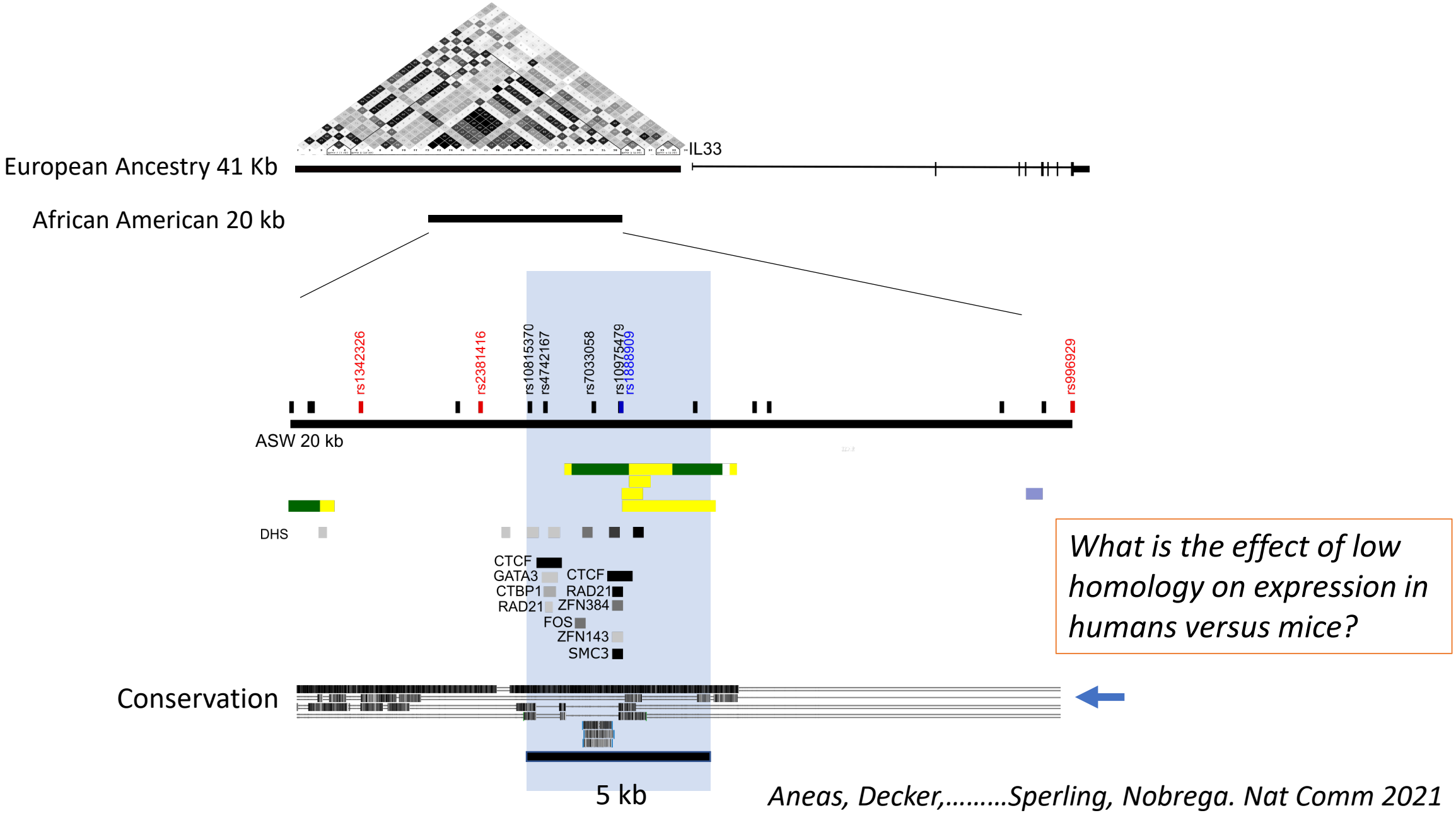
Deletion of the 5kb insulator region eliminates expression in CD31+ MHC II+ microvascular endothelial cells



Deletion of the 5kb insulator region eliminates hIL-33-reporter expression in tracheal basal epithelium and SMG



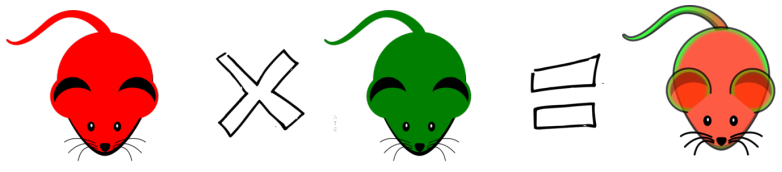
SNPs associated to increased asthma risk are located upstream *of the IL33* gene



What is the effect of low homology on expression in humans versus mice?

Aneas, Decker,……Sperling, Nobrega. Nat Comm 2021

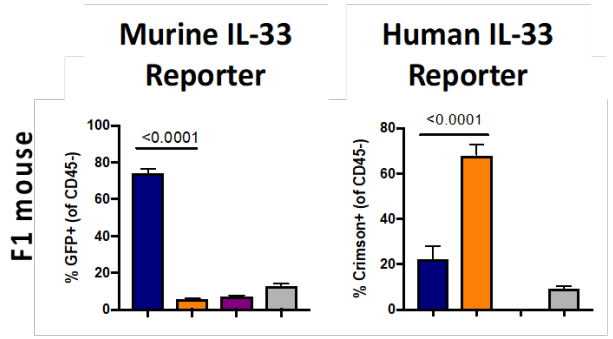
GFP (murine *Il33* reporter) and Crimson (human *IL33* reporter) are expressed by distinct populations in the lungs

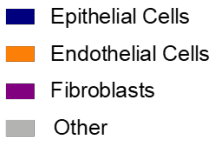


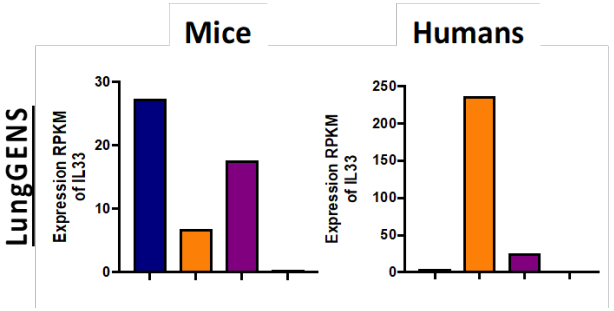
BAC human IL-33: Crimson reports human IL-33

IL-33$^{GFP/GFP}$: GFP reports murine IL-33

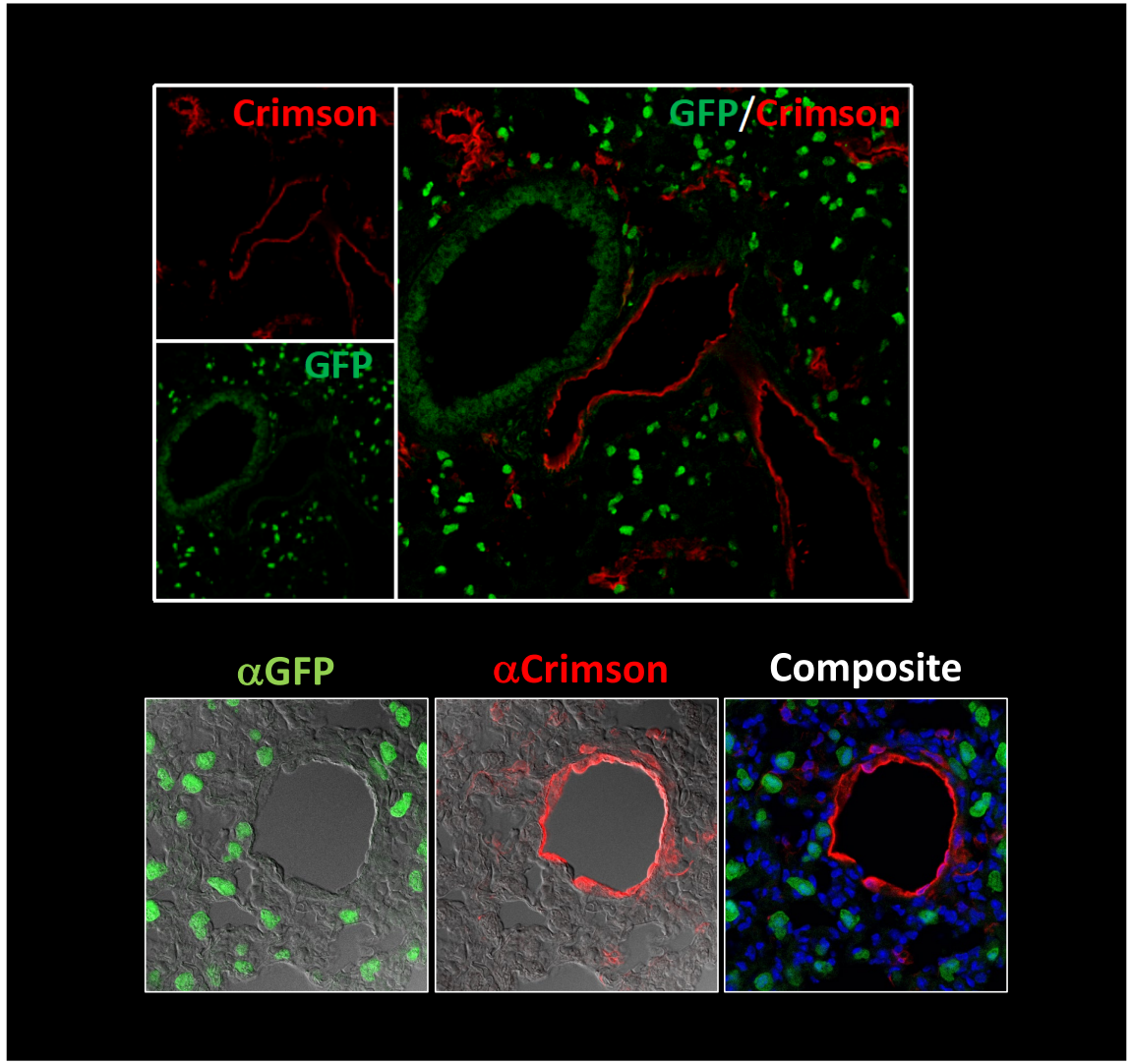
F1: Dual Crimson and GFP reporter mouse



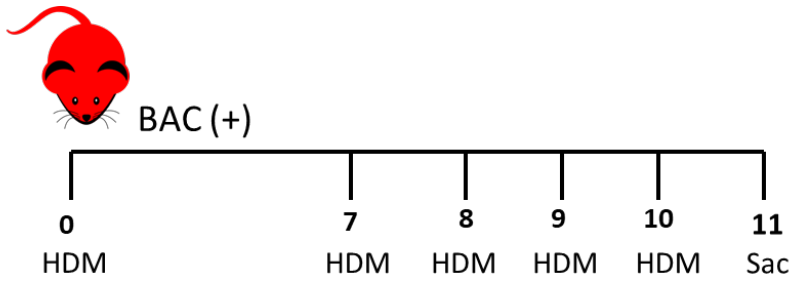




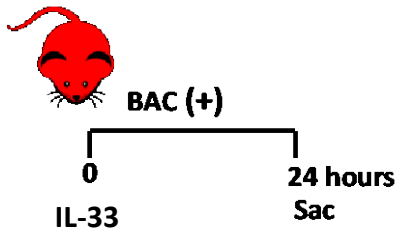
Lungs

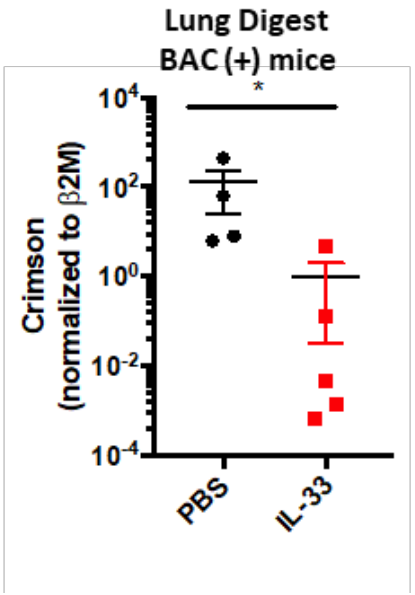


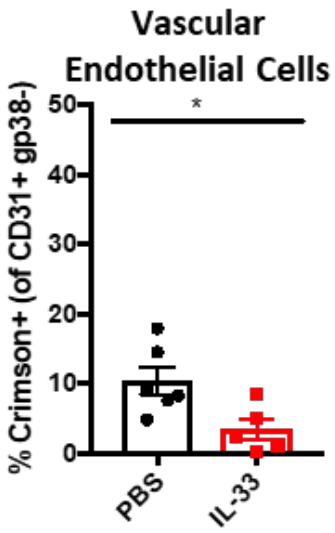
Allergen sensitization and challenge *decreases* human-IL-33 reporter expression, but not murine IL-33 reporter expression

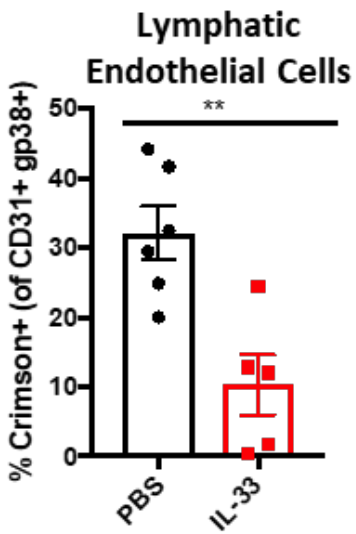


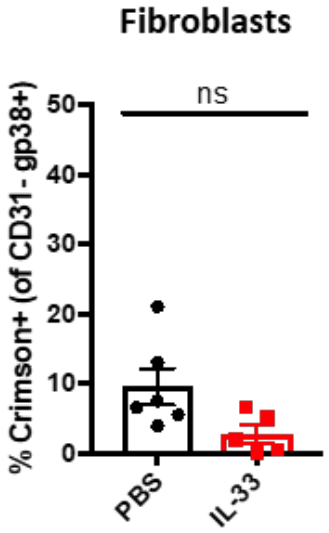
IL-33 *decreases* human IL-33-reporter expression in endothelial cells suggesting a negative feedback loop

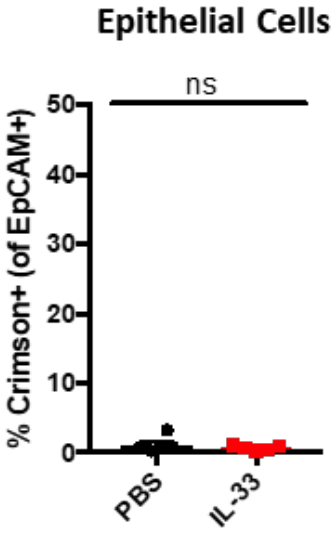




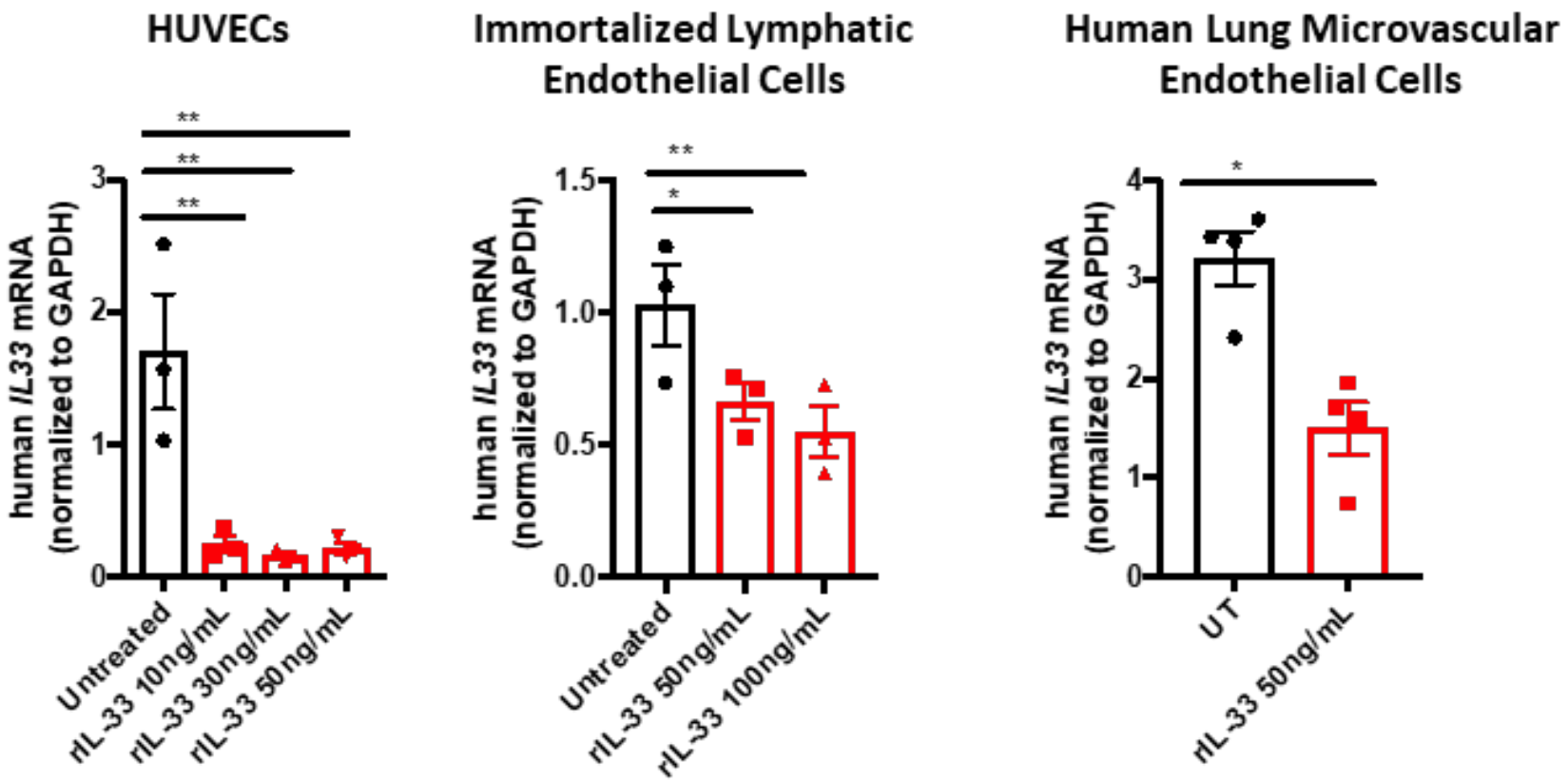








IL-33 *decreases IL33* mRNA expression in endothelial cells suggesting a negative feedback loop



Conclusions

- We have identified a 5kb region that confers tissue-specific expression of IL33
- The 5kb region has insulator activity and contains GWAS SNPs that affect its function
- Human IL-33 is primarily expressed in *endothelial* cells, not epithelial cells as in mice.
- IL-33 negatively regulates its own expression in endothelial cells, and this downregulation is dependent on the 5kb region

Outline

IL-33 is produced by dendritic cells and is involved in allergic sensitization in the lungs to multiple types of allergens

IL-33 levels in Amish farm children are suppressed by their environment,
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IL-33 is produced by different lung cells in humans compared to mice.

Acknowledgements

Donna Decker, Cara Hrusch, Tania Velaz, Tim Kuzel, Trevor Decker, **Dan Camacho**, Phil Verhoef, **Maile Hollinger**, **Rebecca Voogt**, **Chanie Howard**, Kelly Blaine, Paulette Krishack, Young me Yoon,

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Nathan Schoettler
Michelle Stein

Marcus Clark
Domenick Kennedy

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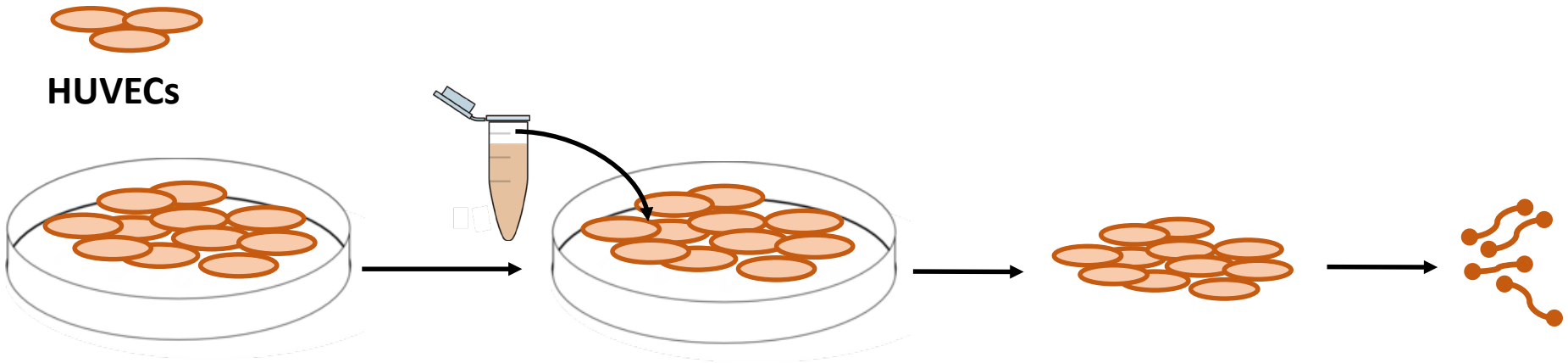
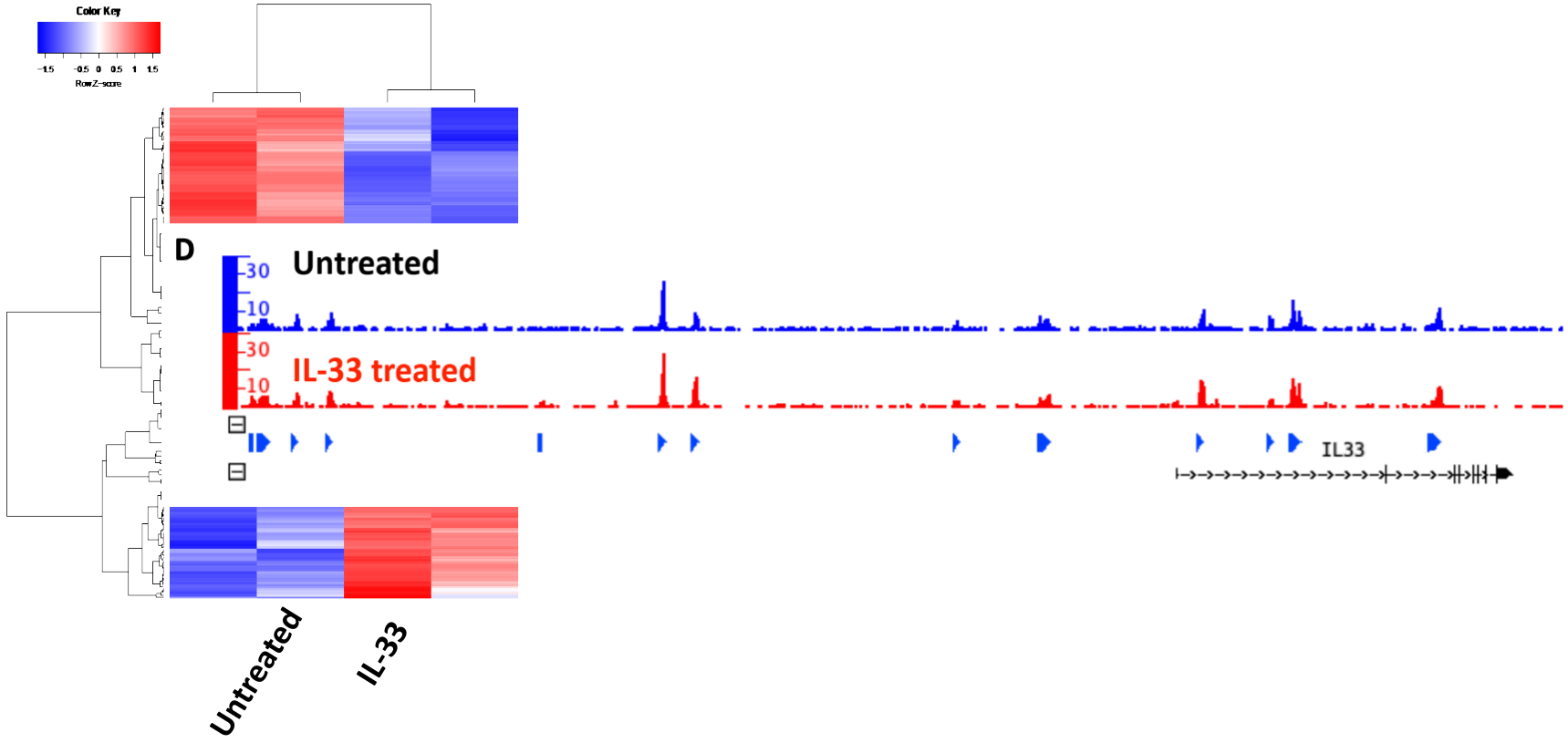
Funded by NHLBI R01, NIAID U19 - and NIAID T32, F31, AHA postdoc

Does rIL-33 downregulate *IL33* transcription by reducing chromatin accessibility at the *IL33* locus?

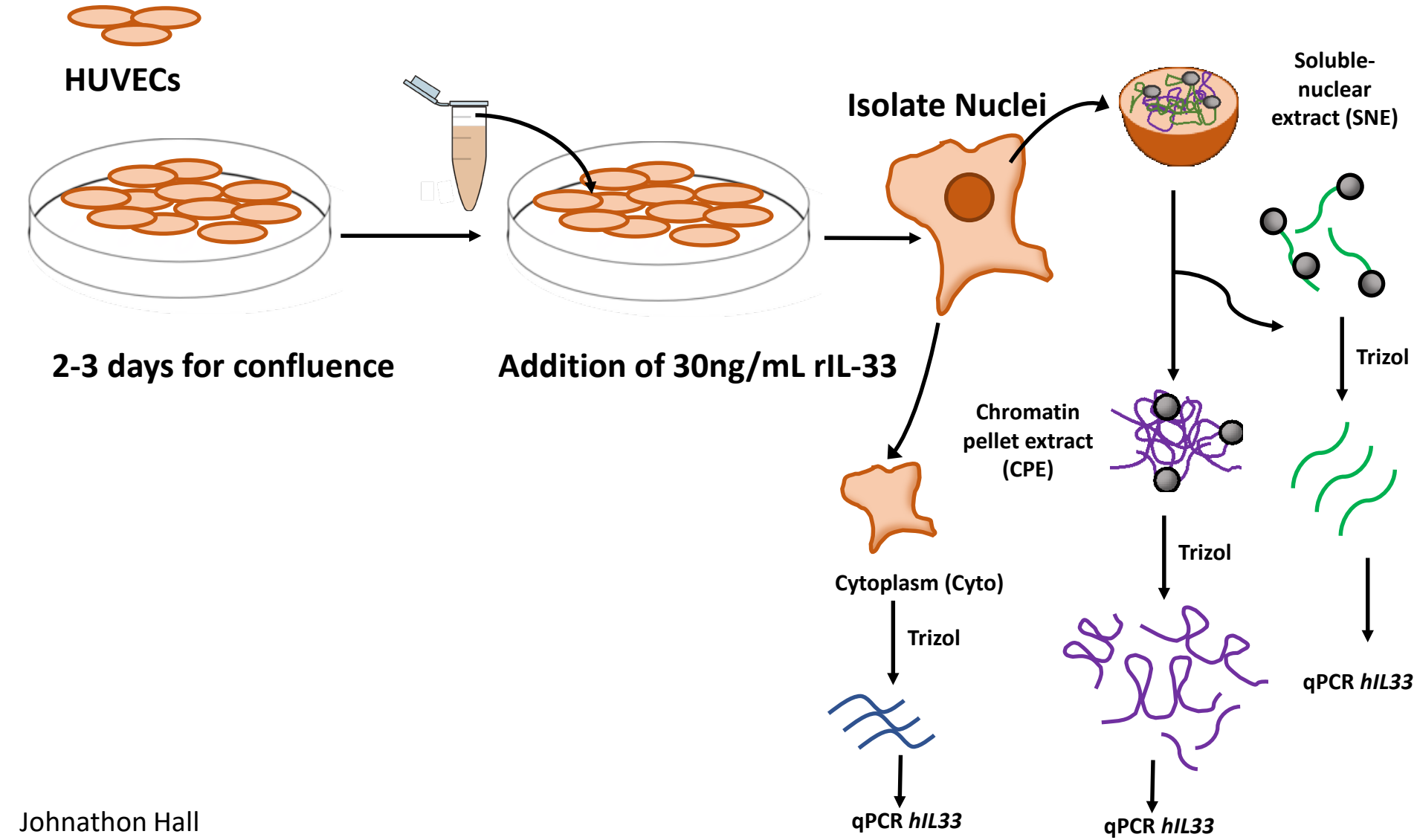


Extracellular IL-33 does not alter accessibility at the *IL33* locus

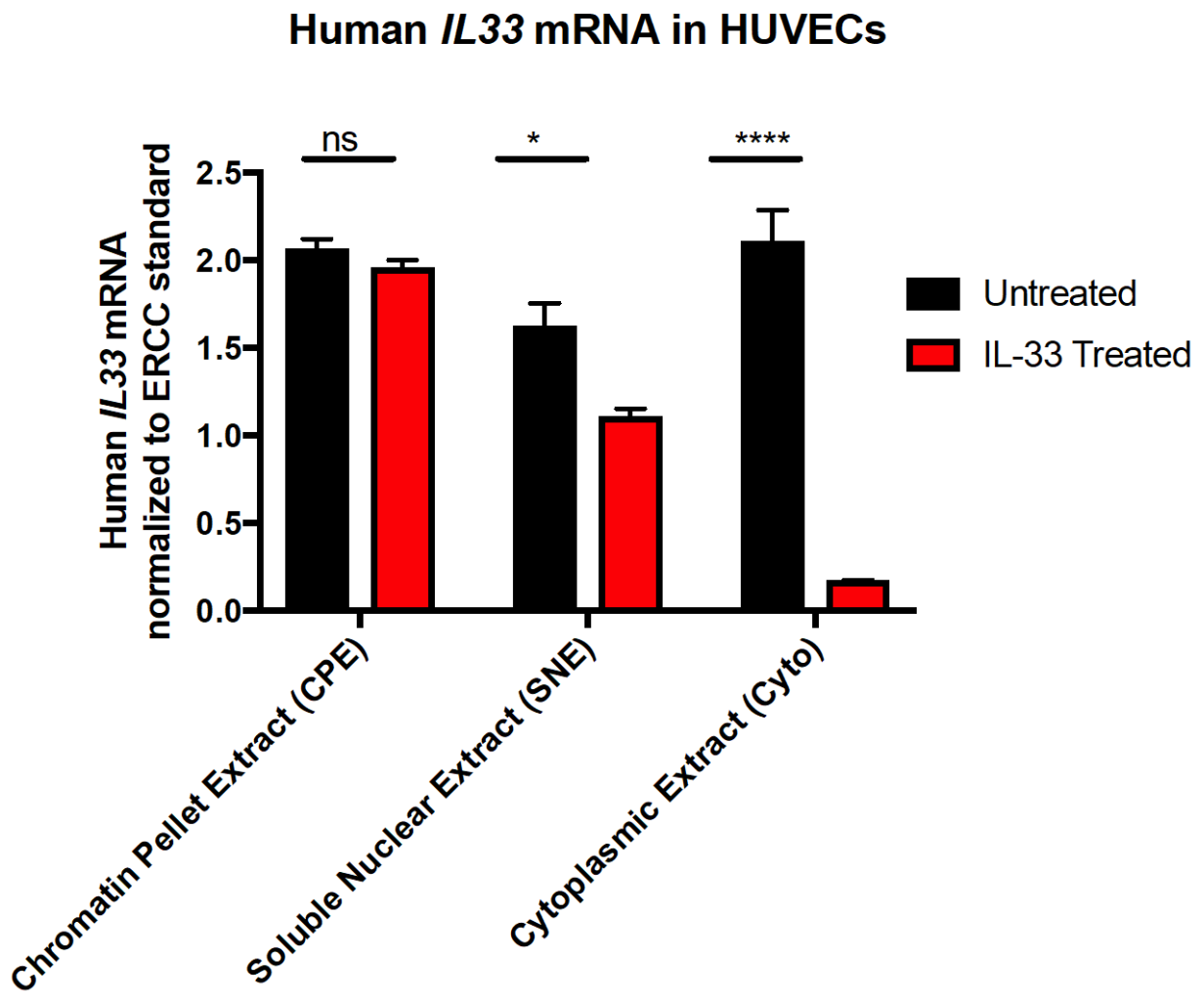


Domenick Kennedy

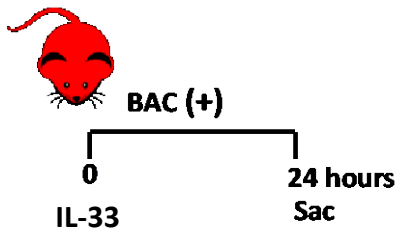
Does extracellular IL-33 increases *IL33* mRNA turnover?

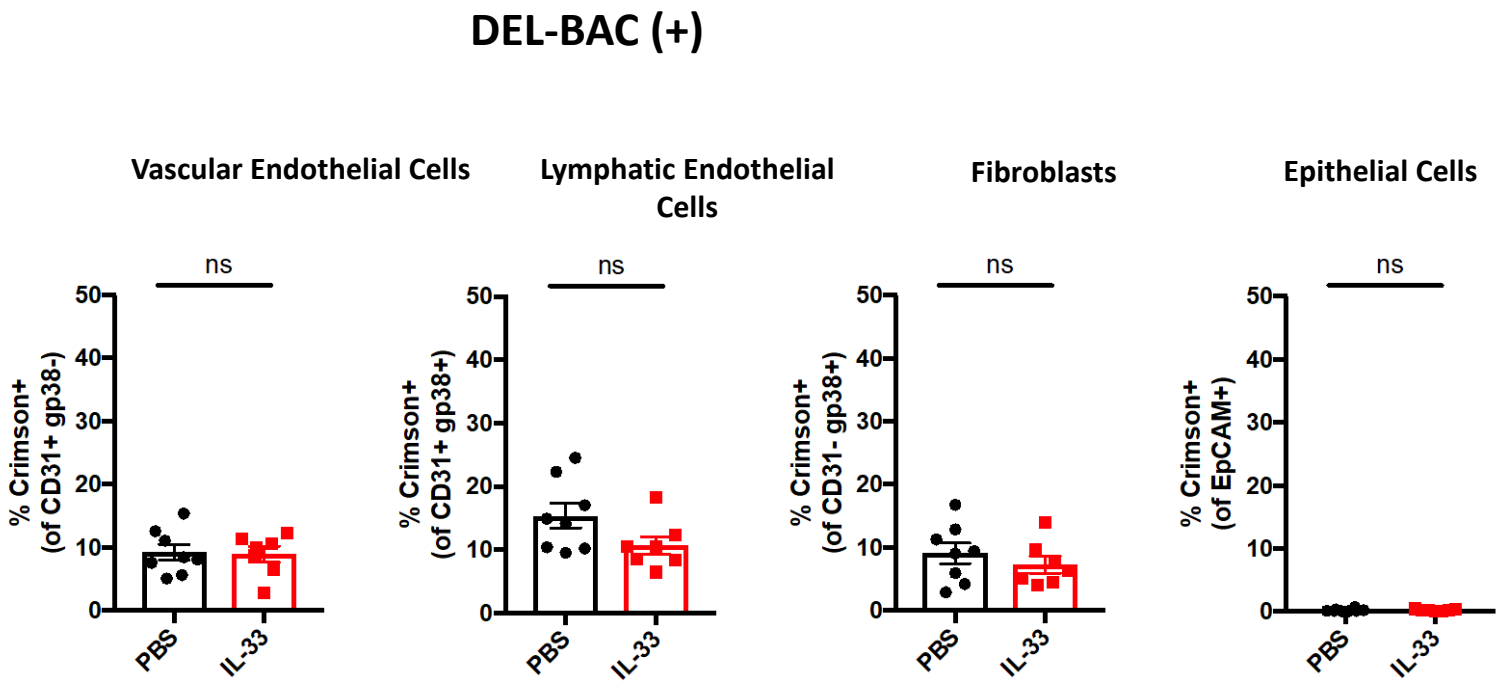


Exogenous IL-33 treatment decreases nuclear *IL33* mRNA stability in human endothelial cells



IL-33 *decrease* of human IL-33-reporter expression is dependent on the 5kb insulator region





Acknowledgements

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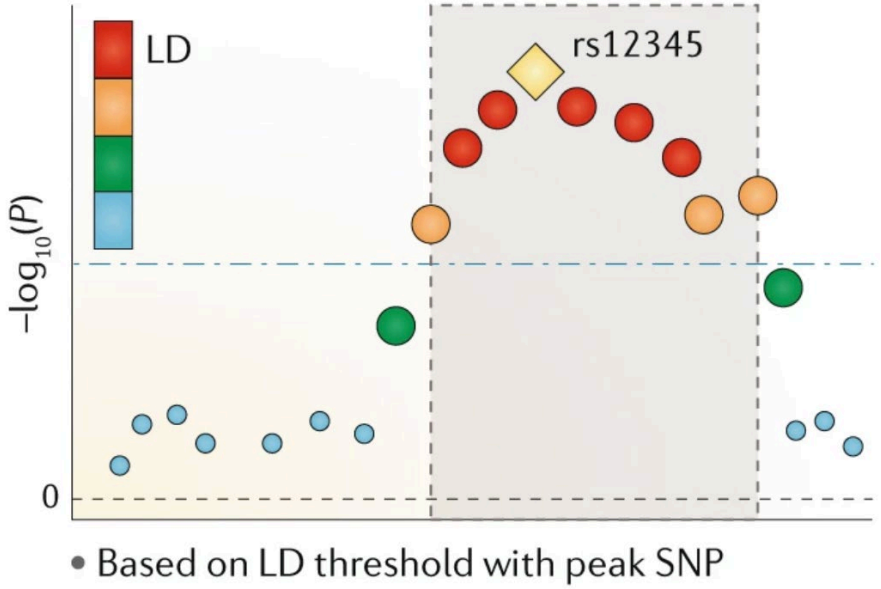
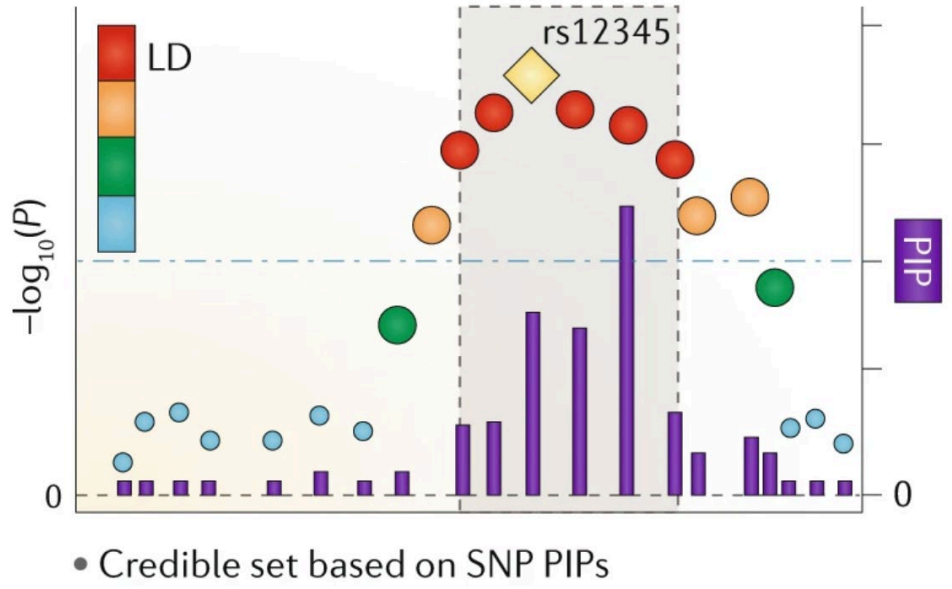
Donna Decker, Cara Hrusch, Anne Sperling, Tania Velaz, Tim Kuzel, Trevor Decker, Dan Camacho
Phil Verhoef, **Maile Hollinger**, **Rebecca Voogt**, **Chanie Howard**, Kelly Blaine, Paulette Krishack, Young me Yoon
Past: Jesse Williams, Hozefa Bandukwala, Bryan Clay, Melissa Tjota, Kathleen Mills

Bayesian fine-mapping quantifies uncertainty in variable selection

Heuristic LD approach



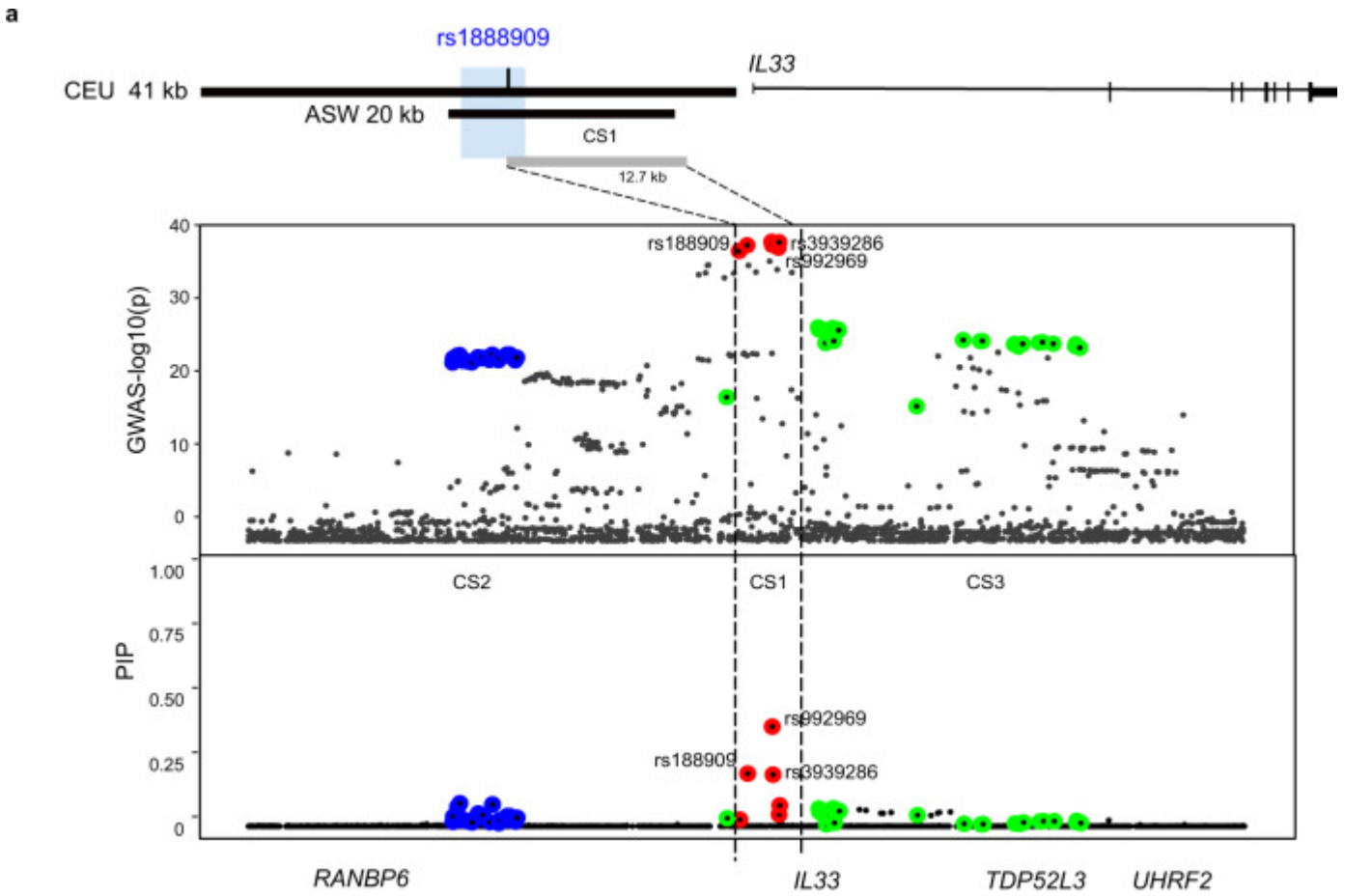
Bayesian fine-mapping



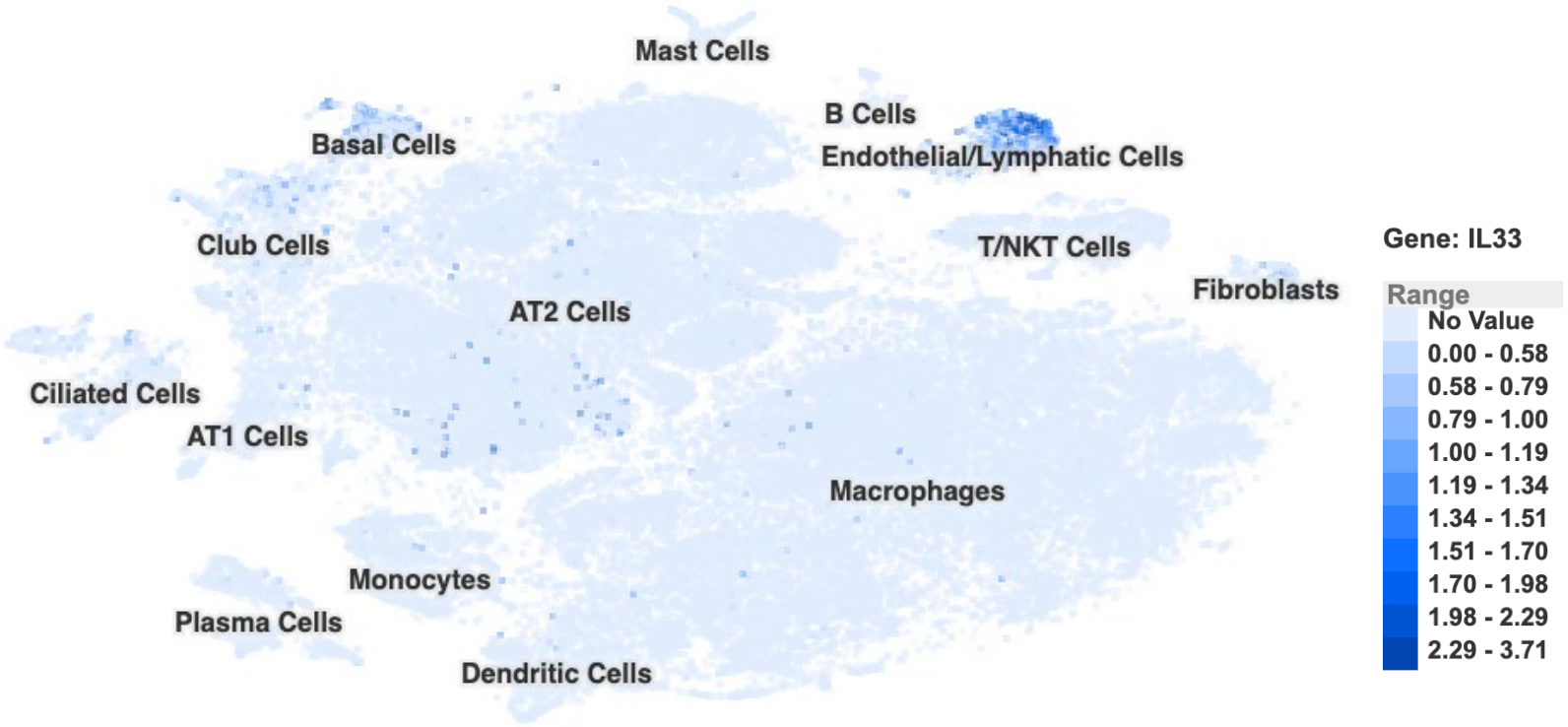
- Heuristic LD approach **deterministically** chooses SNPs above certain LD threshold with the lead SNP
- Limitations: arbitrary threshold, doesn't quantify uncertainty

- Bayesian fine-mapping **probabilistically** evaluates each SNP's causal evidence
- **Posterior inclusion probability (PIP)**: the probability of a SNP being causal
- **95% credible set**: a set of SNPs that has 95% or greater probability of containing at least one causal SNP

Bayesian fine-mapping quantifies uncertainty in variable selection

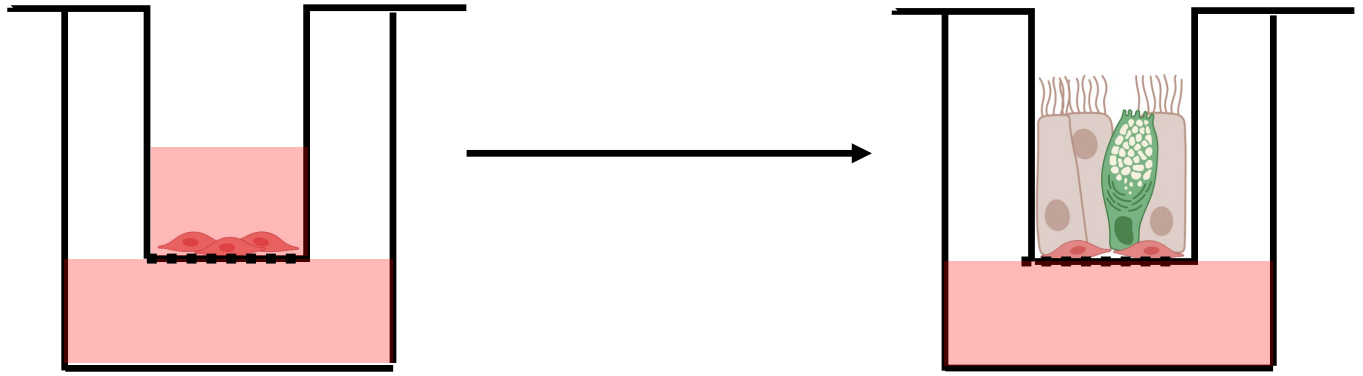


SC-RNAseq demonstrate *IL33* is abundantly expressed in human lungs



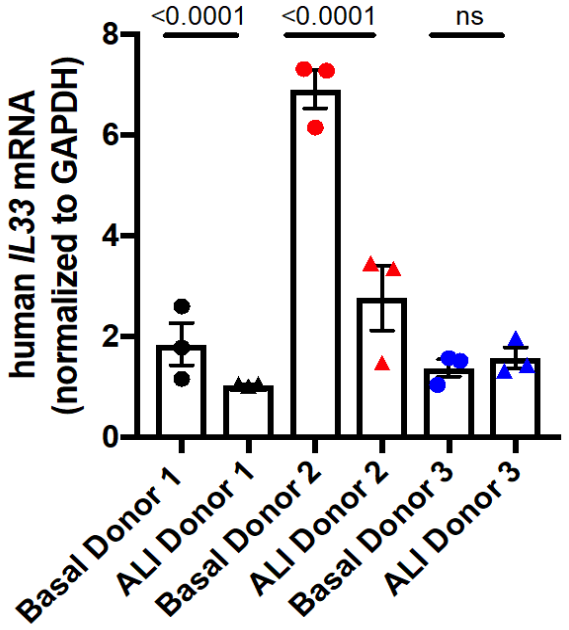
Reyfman *et al.*, 2017

Basal lung cells downregulate *IL33* mRNA as they differentiate in ALI epithelial cultures *in vitro*

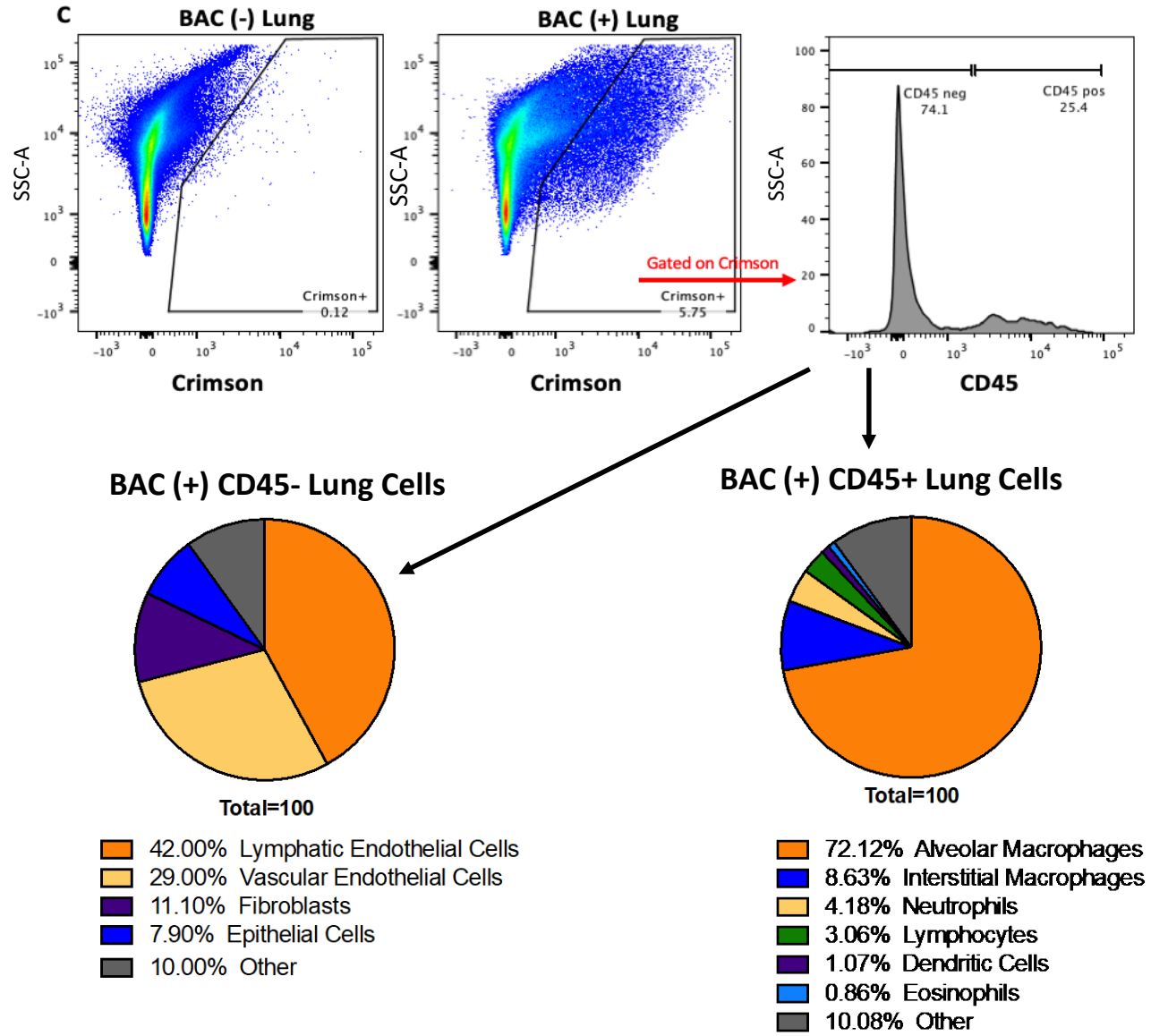


1.0x10^5 cells seeded on Transwell insert

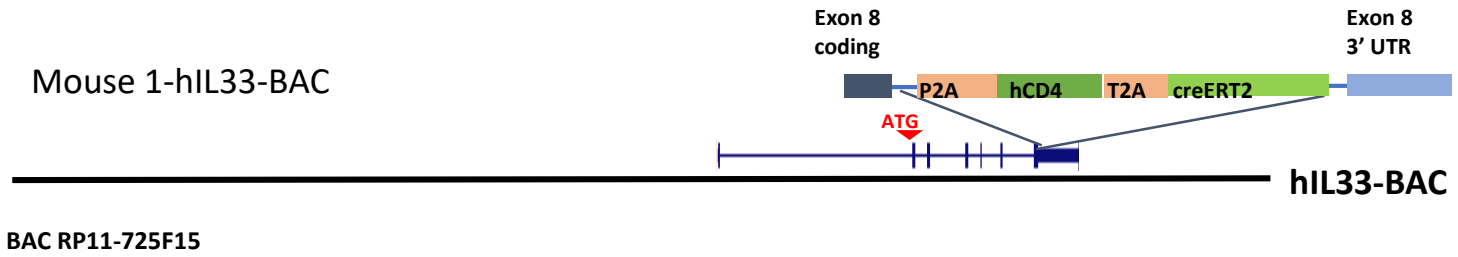
"Air Lift" Media supplied to basal chamber

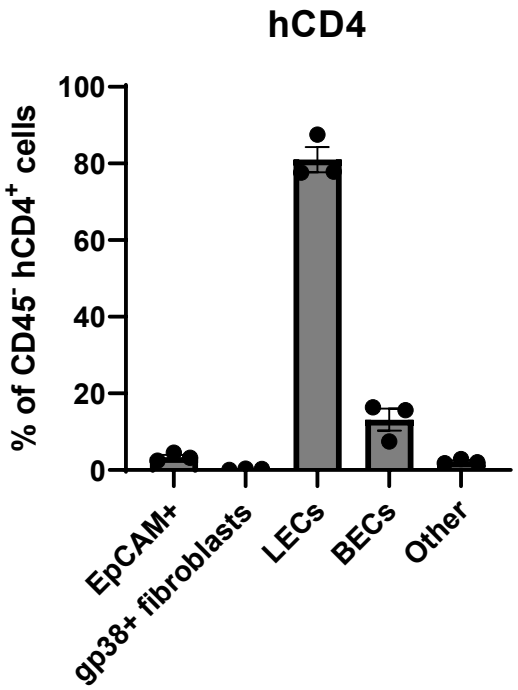


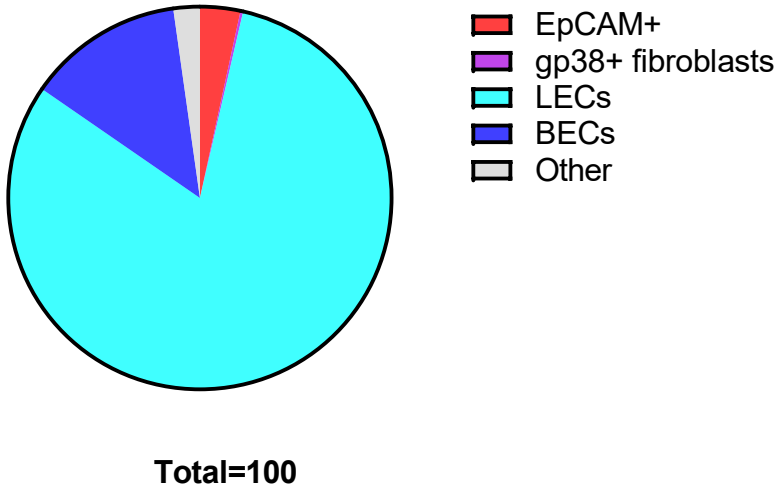
Crimson (human *IL33* reporter) is expressed largely by non-hematopoietic cells in BAC (+) lungs



New BAC Tg mice that make hIL-33

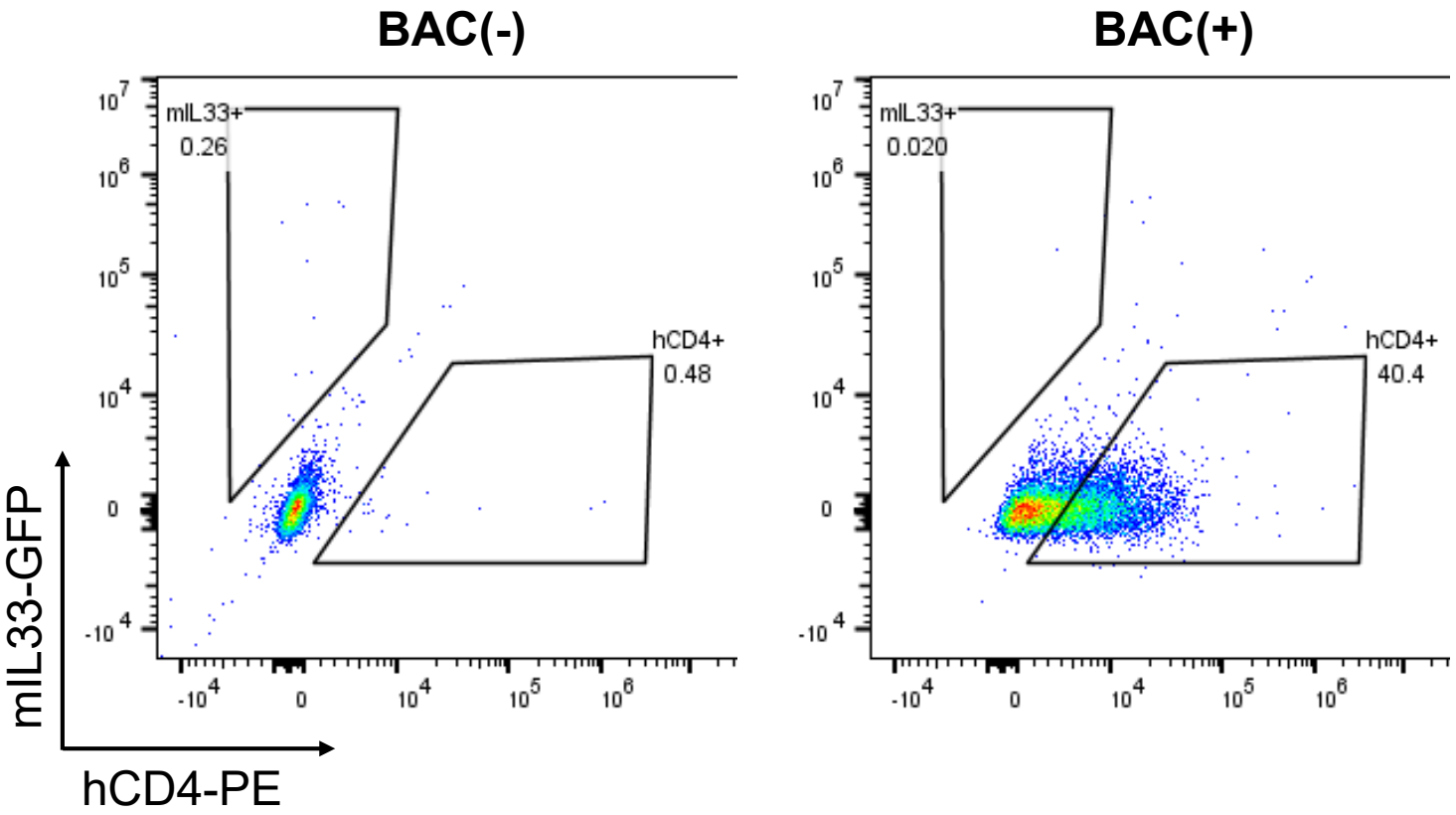


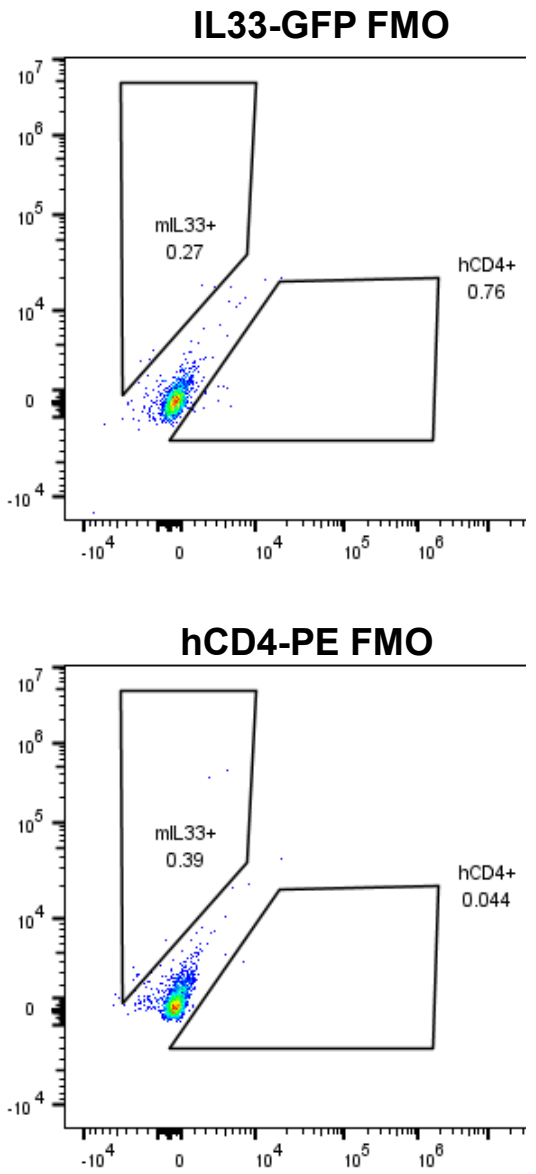




Tissue	Hu CD4 (HuIL-33 reporter)	GFP (Mouse-IL33 reporter)	Double positive?
Epithelial cells	Neg	++++	None
Lym Endo	++++	+	+ (all GFP are also huCD4)
Vasc Endo	+	Neg	None
Fibroblast	Neg	++	None
Neutrophils	++++	Neg	None
Eosinophils	+/-	Neg	Noe
AM	+	Neg	None
Intersitial Macs	+	Neg	None
DC1	++++	Neg	None
DC2	++++	Neg	None
Ly6C+ Mono	+++	Neg	None

Neutrophils





Gated on live, CD45^{+}, Lin (CD3/CD19/NK1.1)$^{-}$ cells

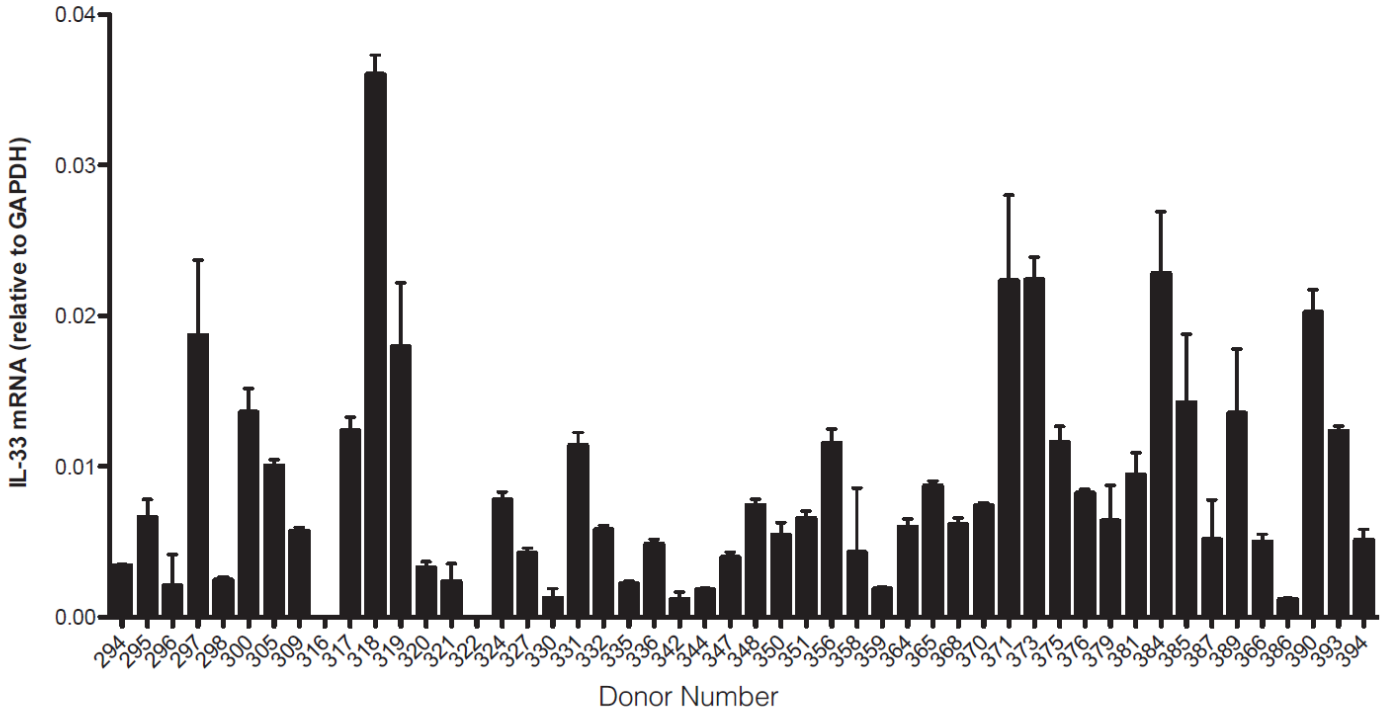


Figure 4.1. *IL33* is expressed in the hematopoietic cells of human pulmonary tissue and varies widely between individuals. qPCR for *IL33* levels in the d0 RNA isolates from the human pulmonary hematopoietic cells of 50 lung donors.Values are

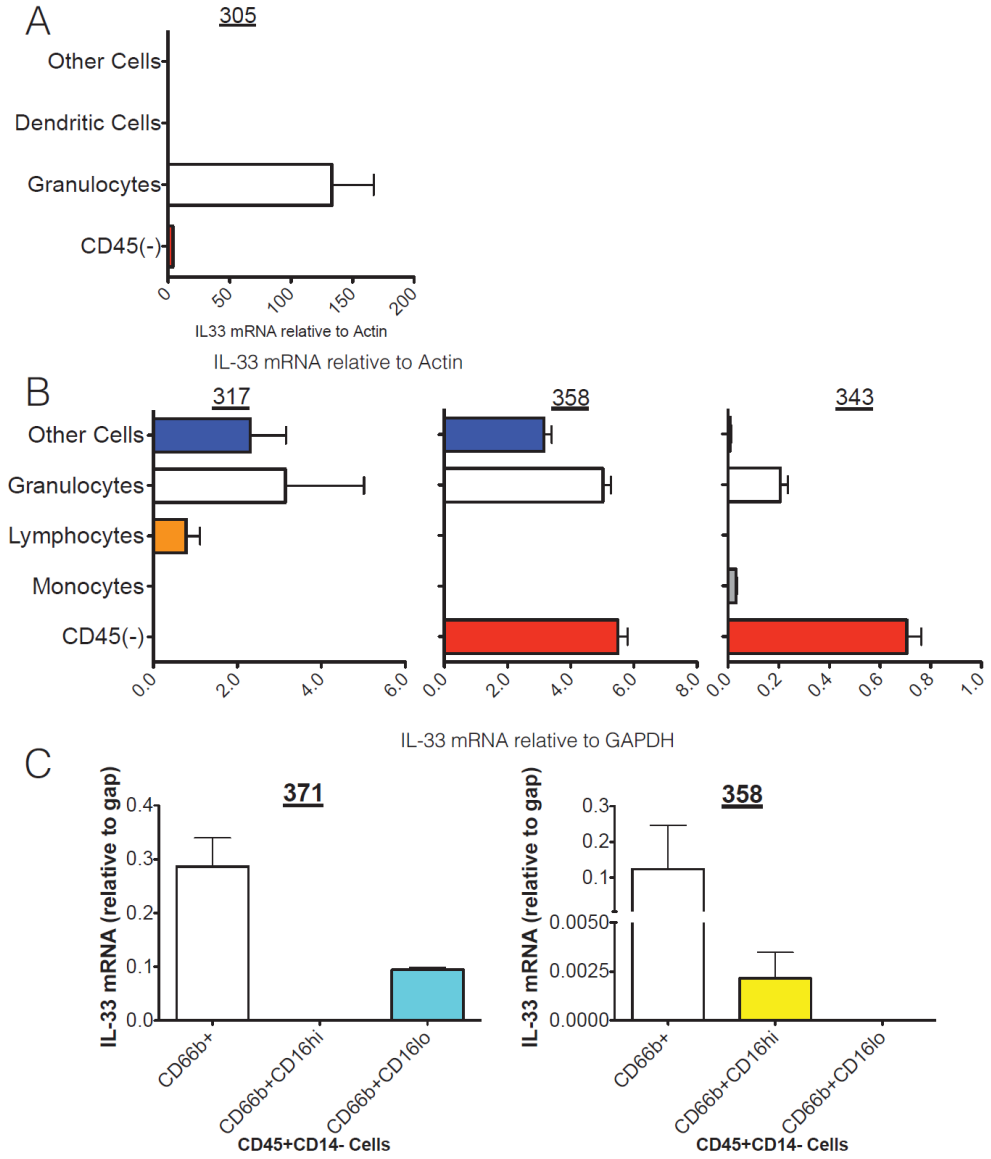


Figure 4.10. *IL33* is produced by a CD66b+ granulocytic population of lung cells. Distinct cell populations from human lungs were isolated by Flow Cytometric Sorting (FACS), from which cDNA was prepared (Continued on page 96).

Figure 4.10, continued. (A) R305 was sorted into four broad populations based on the following phenotypes: Red: CD45-, White: Granulocytes (CD45+CD66b+), Green: Dendritic Cells (CD45+CD11c+), and Blue: Other Cells (CD45+CD11c-CD66b-). *IL33* levels are shown relative to *ACTB*. (B) R317, R358, and R343 were sorted base on the following phenotypes: Red: CD45-, Grey: Monocytes and macrophages (CD45+CD14+), Orange: Lymphocytes (CD45+CD14-CD3/19+CD66b-), White: Granulocytes (CD45+CD14-CD3/19-CD66b+), and Blue: Other Cells (CD45+CD14- CD3/19-CD66b-). *IL33* levels are relative to *GAPDH*. (C) R371 and R358 were sorted based on the following phenotypes: White: All granulocytes (CD45+CD14-CD3-CD19-CD66b+), Yellow: CD16hi granulocytes (CD45+CD14-CD3-CD19-CD66b +CD16hi), Cyan: CD16lo granulocytes (CD45+CD14-CD3-CD19-CD66b +CD16lo).