Known & novel regulators of itch cytokine IL-31 in type 2 disease

Marlys Fassett, MD PhD; Associate Professor of Dermatology

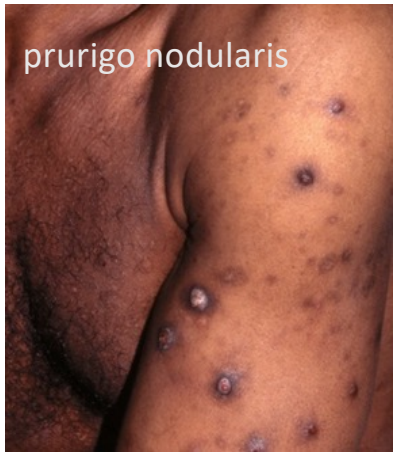
Learning Objective:
To learn about nodes that alter IL-31 expression and IL-31+ cell biology in type 2 inflammatory diseases

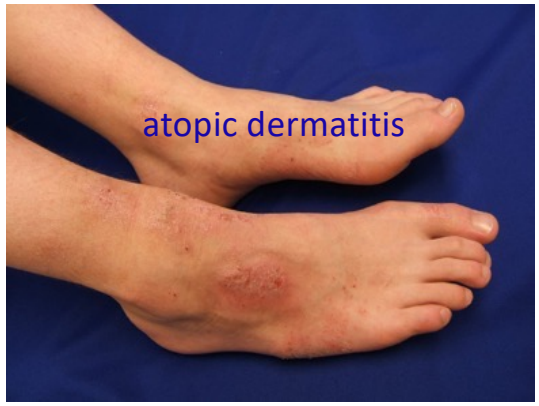
No Conflicts of Interest to declare
Contact: marlys.fassett@ucsf.edu

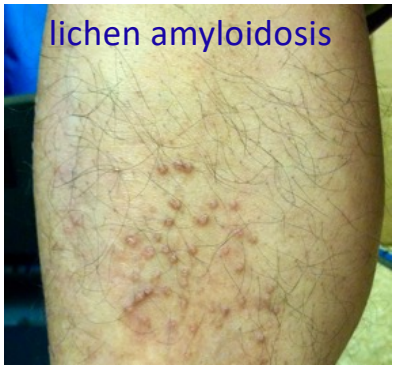
Known & novel regulators of itch cytokine IL-31 in type 2 disease

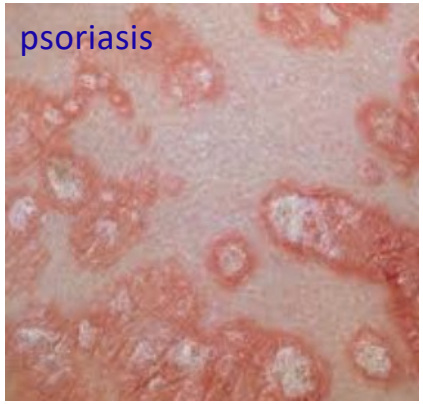
Marlys Fassett, MD PhD; Associate Professor of Dermatology
International Society of Atopic Dermatitis, October 2025

IL-31 IS DETECTABLE IN PATIENTS WITH SEVERELY ITCHY CHRONIC INFLAMMATORY SKIN CONDITIONS OF DIVERSE PATHOPHYSIOLOGY



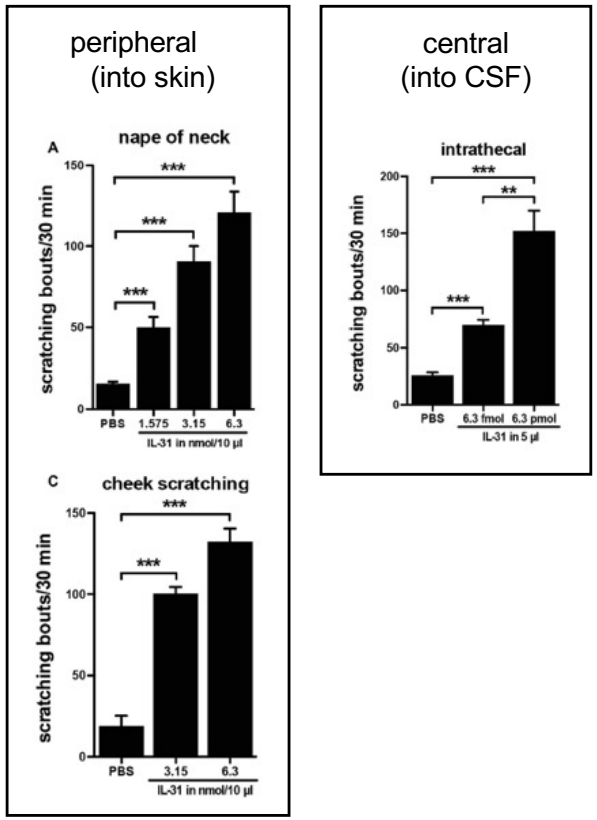






IL-31’S CAPACITY TO TRIGGER ITCH SENSATION (SCRATCHING) IS UNAMBIGUOUS

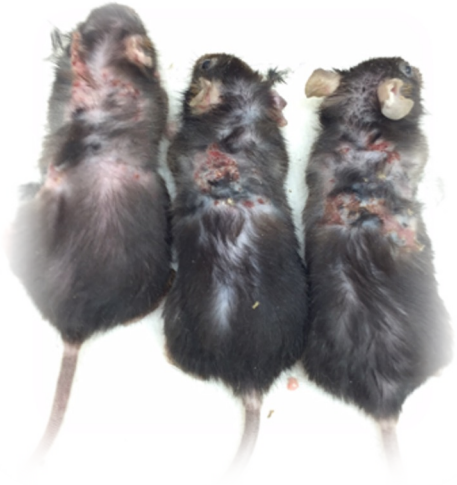
Shimada & Lamotte. Pain 2008;139(3):681-7.



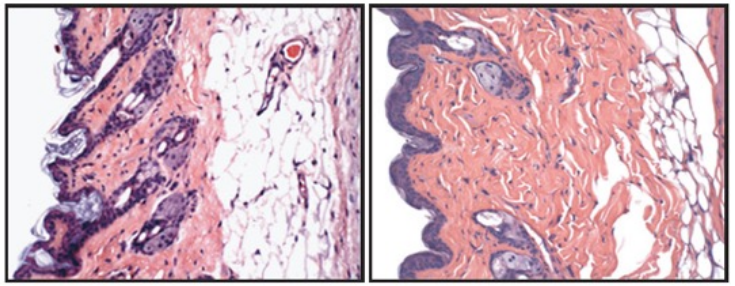
Cevikbas *et al.* J Allergy Clin Immunol 2014;133:448-60.

IN MICE, *IL31* OVEREXPRESSION CAUSES OBVIOUS SKIN PHENOTYPES: ITCHING/SCRATCHING, DERMAL FIBROSIS, INFLAMMATORY INFILTRATES

Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice

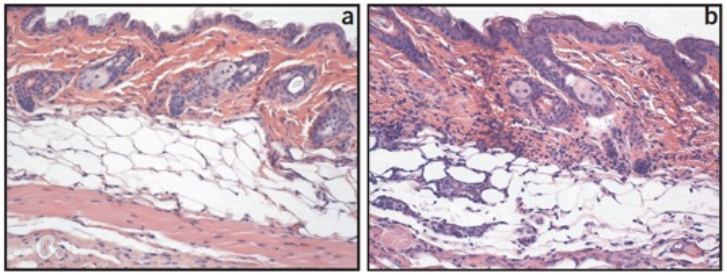


B6 B6.Il31Tg



< acanthosis
< dermal fibrosis

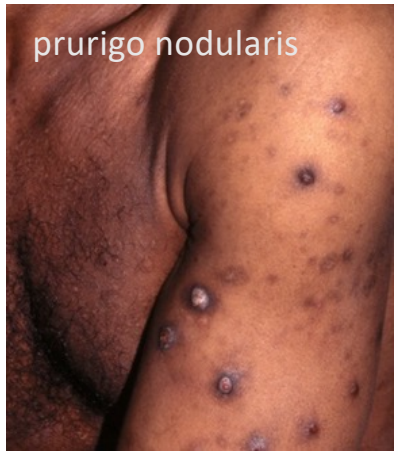
Balb/c Balb/c + 8 days rIL-31

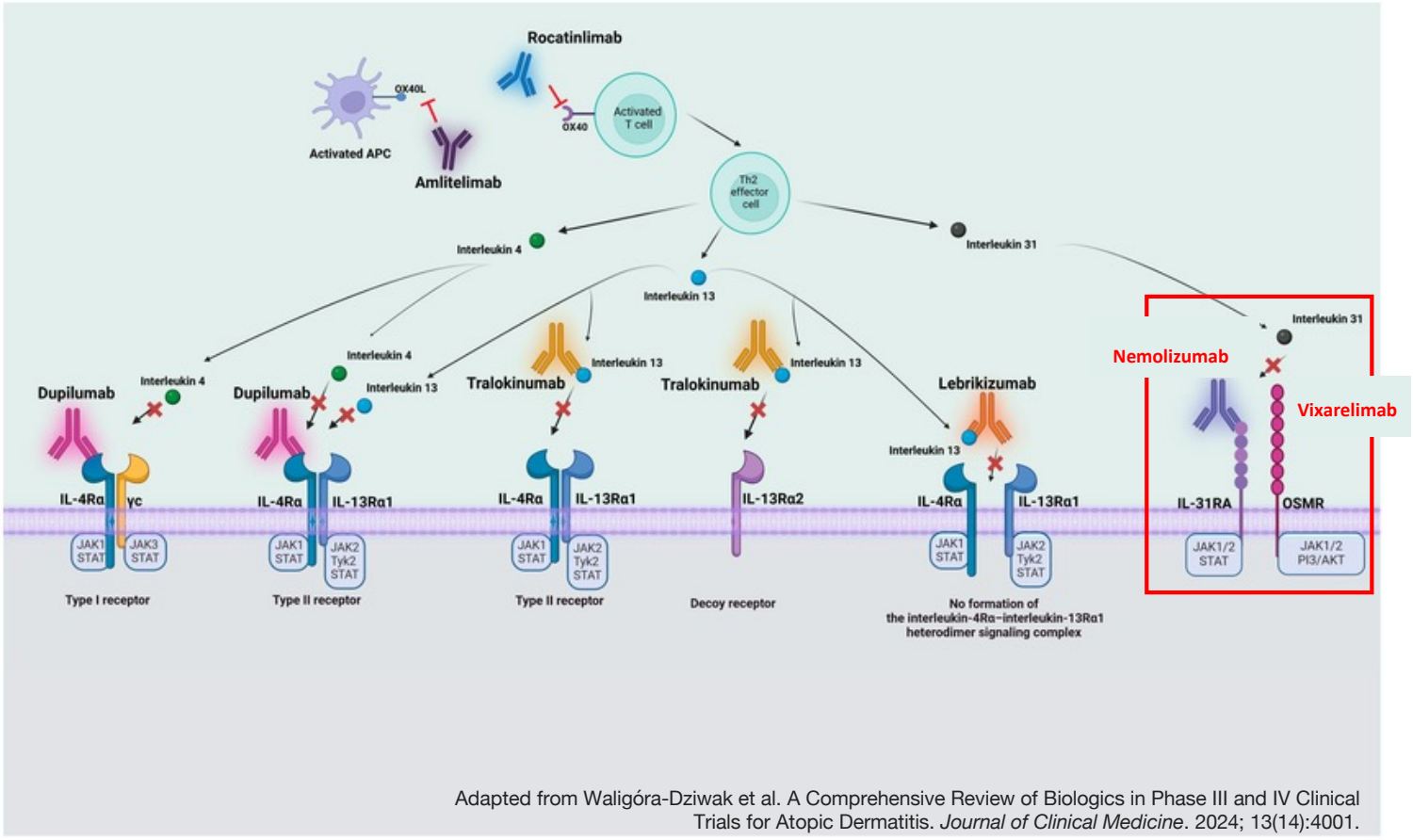


< acanthosis
< inflammatory infiltrates in T2-skewed Balb/c background

Dillon et al. Nat Immunol (2004) 4(7):752-760

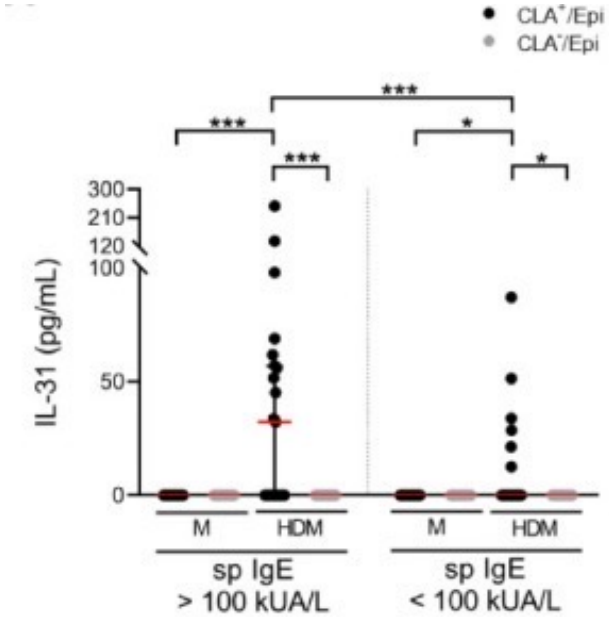
BIOLOGICS THAT TARGET IL-31 RECEPTOR SUBUNITS (IL31RA OR OSMRβ) ARE IN THE CLINIC FOR ATOPIC DERMATITIS AND PRURIGO NODULARIS



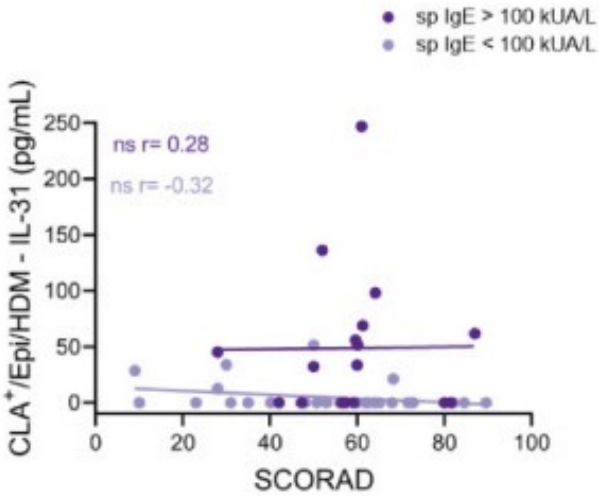


Adapted from Waligóra-Dziwak et al. A Comprehensive Review of Biologics in Phase III and IV Clinical Trials for Atopic Dermatitis. *Journal of Clinical Medicine*. 2024; 13(14):4001.

IN ATOPIC DERMATITIS PATIENT SKIN, IT'S KNOWN THAT MEMORY T CELLS SECRETE IL-31 UPON EXPOSURE TO HOUSE DUST MITE (HDM) ALLERGEN-STIMULATED EPITHELIAL CELLS



Presence of HDM-specific IgE is sufficient, HDM-IgE patients with low or high titer all have IL-31 producing T cells in peripheral blood

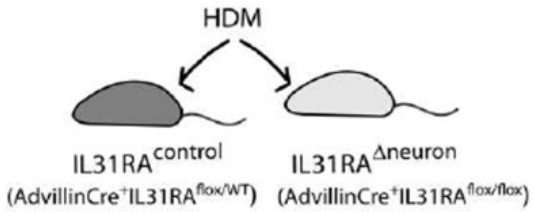


(NB no relationship between IL-31 production and dermatitis severity)

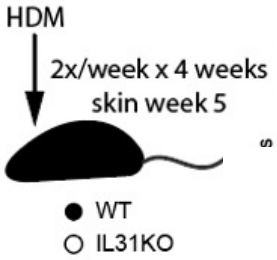
Sans-de San Nicolàs et al. Front Immunol. 2023 Mar 13;14:1124018. doi: 10.3389/fimmu.2023.1124018.

WE BUILT A SET OF TOOLS TO SORT OUT WHAT IL-31 AND ITS RECEPTOR DO IN VIVO IN THE CONTEXT OF VARIOUS INFLAMMATORY DISEASE MODELS

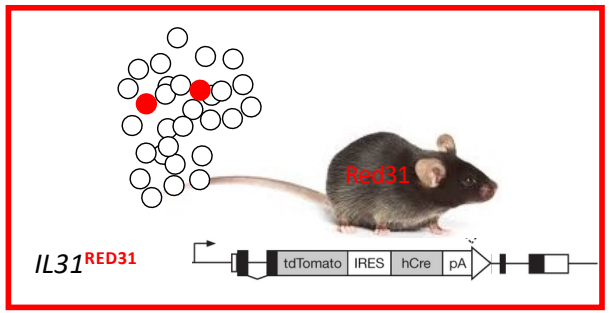
IL31RAKO (receptor knockout, gift from Genentech)
IL31RAflox (conditional receptor knockout; published 2023*)



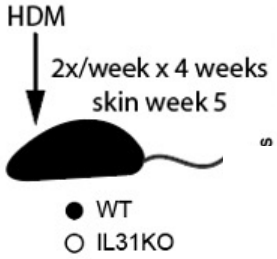
IL31KO (cytokine global knockout)
IL31flox (conditional knockout; not published)

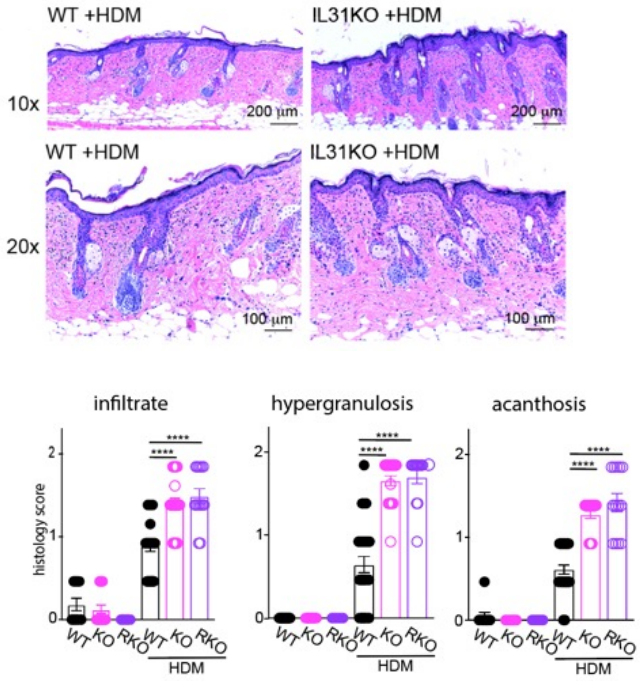


Red31 (knockin/knockout RFP reporter transgene, not published)

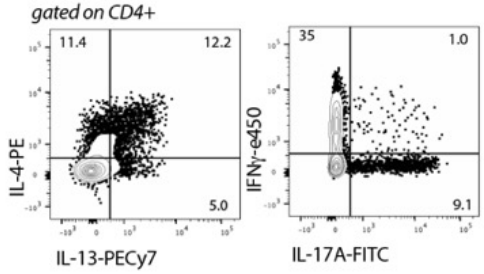


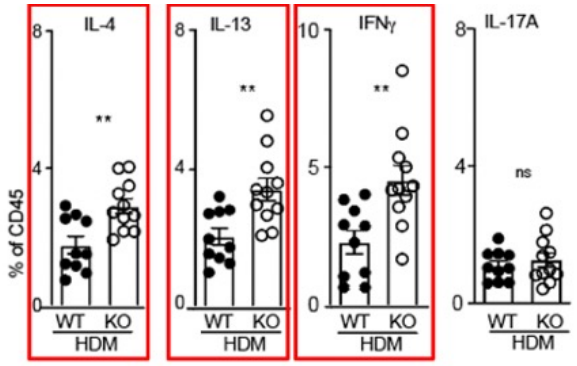
IN IL31KO MICE, IN AN HDM DERMATITIS MODEL, WE “PARADOXICALLY" FOUND SEVERAL METRICS OF TYPE 2 INFLAMMATION INCREASED





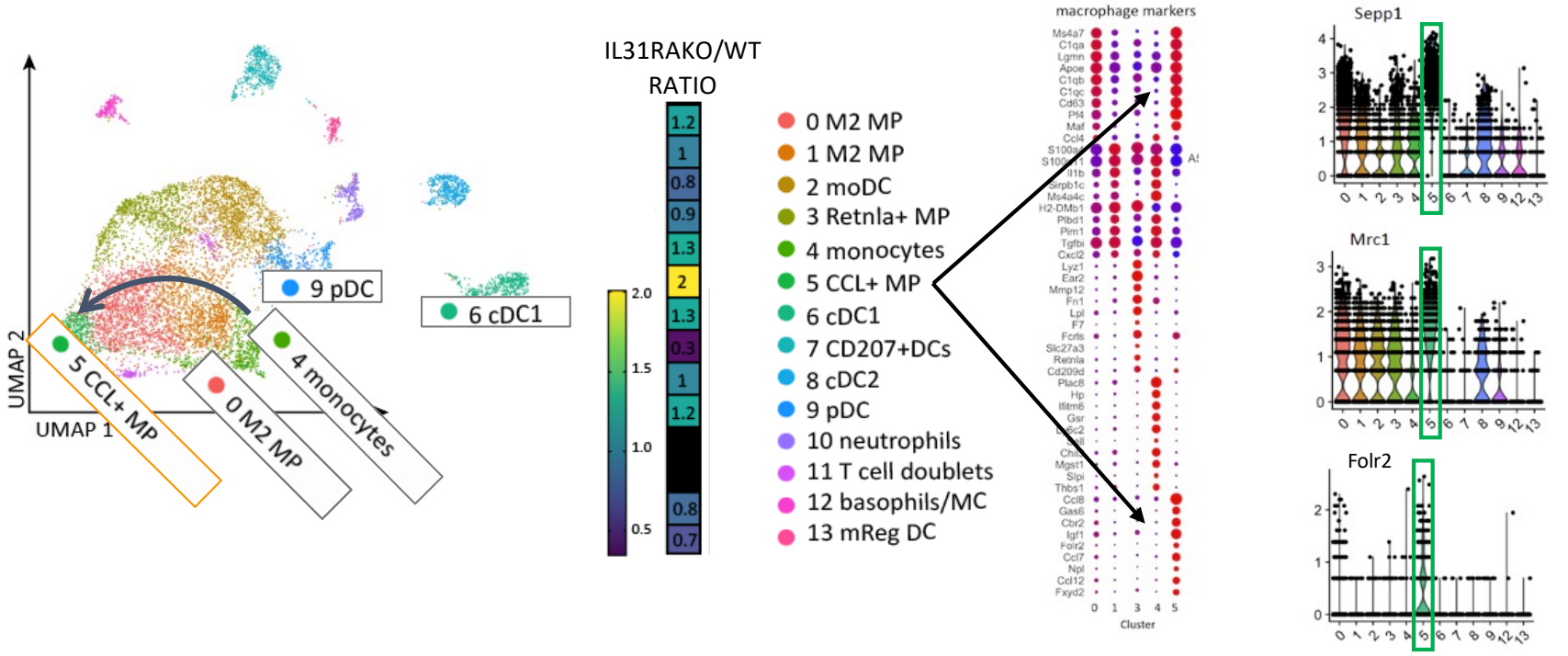
Skin inflammatory markers increased in IL31KO and IL31RAKO:
Epidermal acanthosis and granular layer expansion dermal inflammatory infiltrates





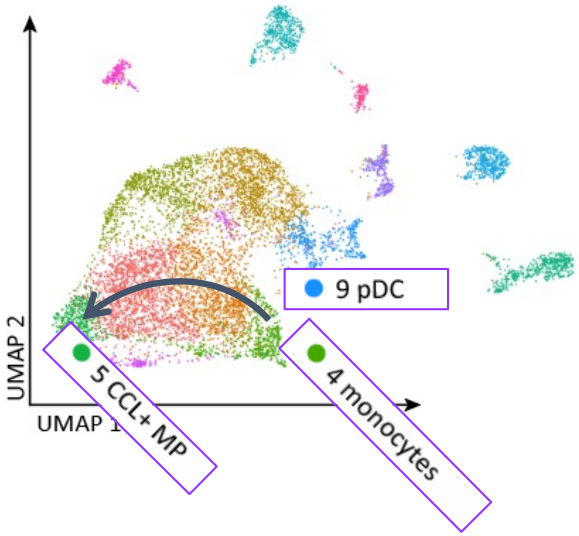
The proportion of effector cytokine-producing skin CD4 T cells also increased

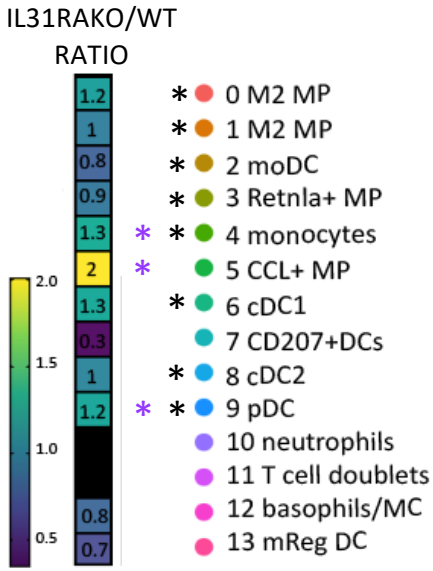
scRNAseq HIGHLIGHTED INCREASED TYPE 2 SKIN INFLAMMATION IN THE MYELOID COMPARTMENT TOO: 2-FOLD EXPANSION OF PHAGOCYTIC TYPE 2 MACROPHAGES, KO>WT



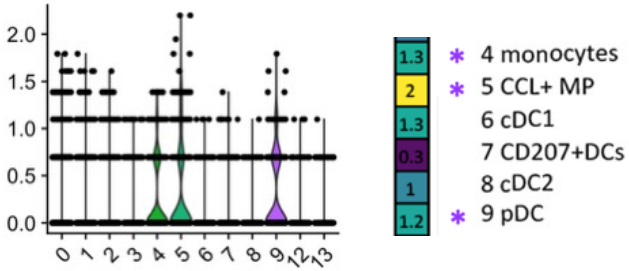
Fassett *et al.*, *Sci. Immunol.* **8**, eabi6887 (2023) 13 October 2023

NOTABLY, THE MYELOID CLUSTERS ENRICHED IN THE KO HDM SKIN WERE IL-4/13 RESPONSIVE (IL4RA+)

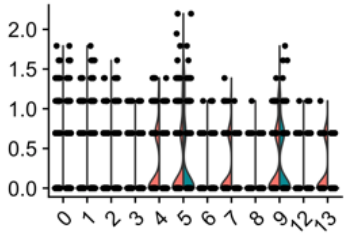




IL4RA WAS EXPRESSED IN THE SPECIFIC MYELOID CLUSTERS THAT EXPANDED IN IL31KO>WT, MARKED WITH *



IL4RA EXPRESSION IN SOME CLUSTERS (4, 7, 13) WAS ENTIRELY ATTRIBUTABLE TO IL31KO CELLS (NOT WT)



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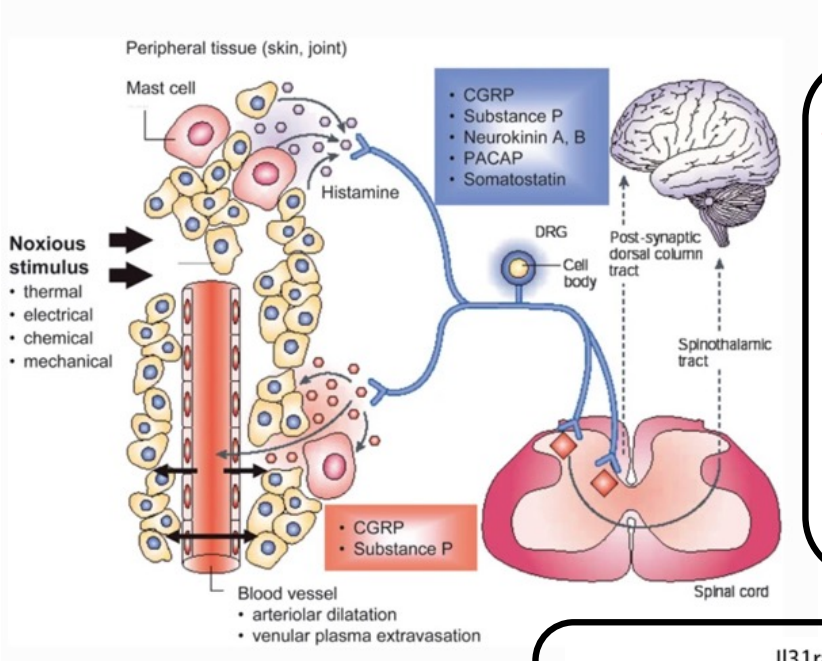
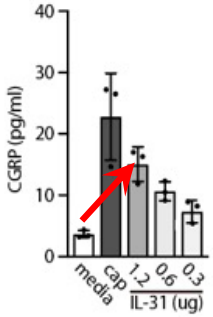
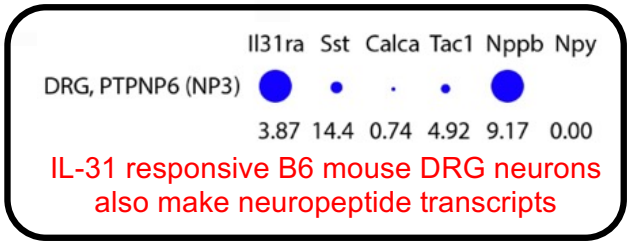
ONE POSSIBLE EXPLANATION FOR IL31KO SKIN PHENOTYPE WAS NEUROGENIC INFLAMMATION: SIGNALING DRIVEN BY NEUROPEPTIDE RELEASE FROM PERIPHERAL AXONS



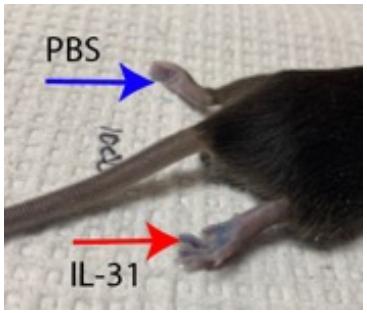
recombinant IL-31 triggered CGRP release from 24h DRG neuron cultures

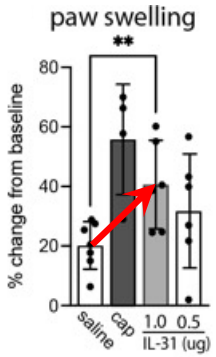


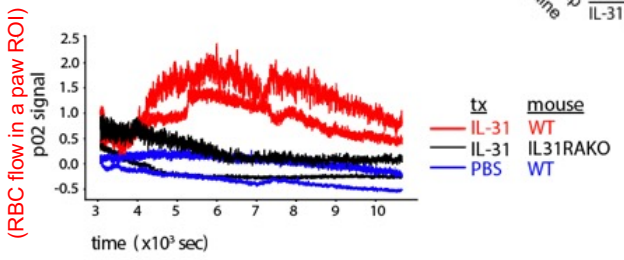


IL-31 responsive B6 mouse DRG neurons also make neuropeptide transcripts

rIL-31 also elicited neuropeptide-dependent vascular changes reflective of CGRP activity (paw swelling, vessel dilatation) but not SP (vascular leak)







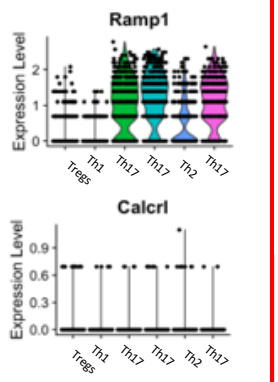
Xiaobing Yu, Juan Salvatierra, Jaela Caston

Fassett *et al., Sci. Immunol.* **8**, eabi6887 (2023) 13 October 2023

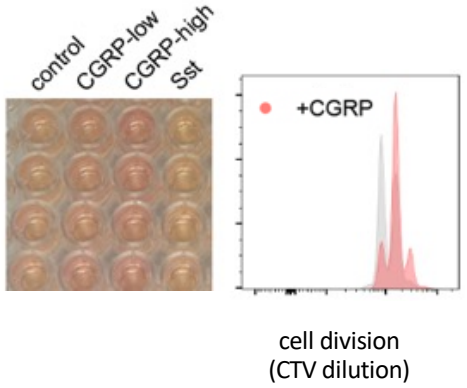
FINALLY, WE MEASURED CGRP-DEPENDENT NEGATIVE REGULATION OF TYPE 2 CD4 T CELL FUNCTIONS *IN VITRO*

CGRP exposure reduces CD4 T cell cycling and IL-13 production

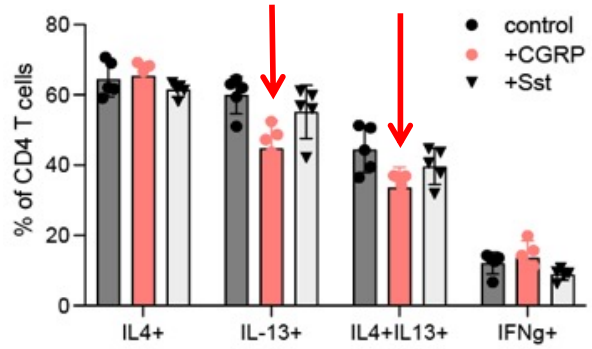
***various SKIN* CD4 T cells express CGRP receptors**



CGRP slows T cell proliferation *in vitro*

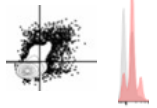


CGRP blunts IL-13 production *in vitro*

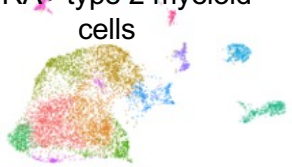


MODEL: IL31RA/OSMRβ+ neurons release CGRP

type 2 CD4 T cell cycling, effector cytokine production



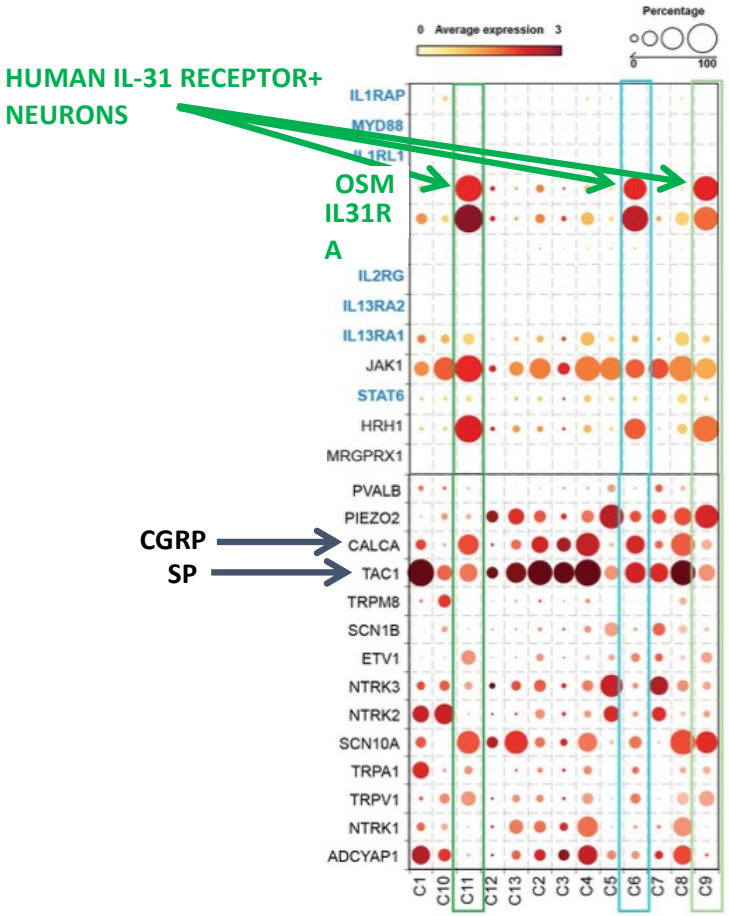
RESULTING IN FEWER IL4RA+ type 2 myeloid cells



THESE EXPERIMENTS CONFIRMED CGRP CAN LIMIT TH2 CELL PROLIFERATION & IL-13 PRODUCTION (IL31/IL31RA BLOCKADE WOULD TAKE THE BRAKES OFF THIS NEGATIVE REGULATORY PATHWAY)

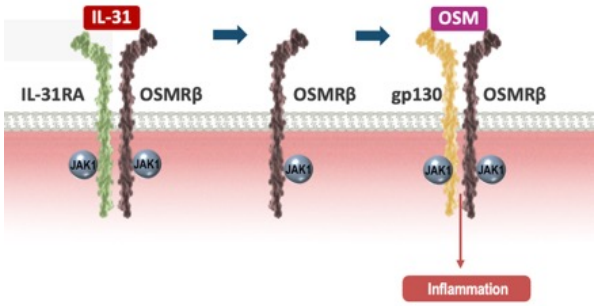
Fassett *et al.*, *Sci. Immunol.* **8**, eabi6887 (2023) 13 October 2023

CADAVERIC HUMAN IL31RA+ SENSORY NEURONS ALSO EXPRESS NEUROPEPTIDES., SUGGESTING POSSIBILITY OF A CONSERVED MECHANISM IN PEOPLE

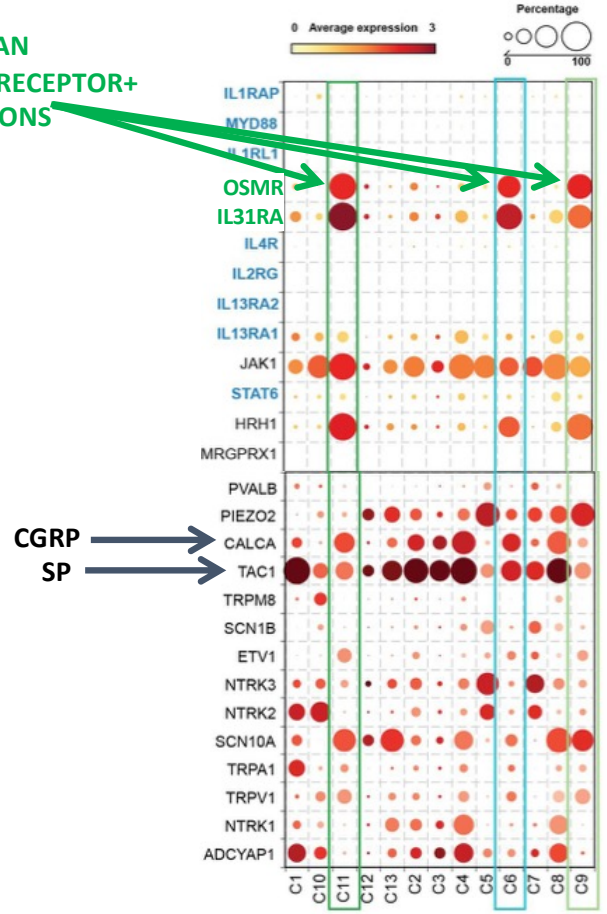


Mack, M et al. Type 2 cytokines sensitize sensory neurons to itch-associated stimuli (2023). https://doi.org/10.3389/fnmol.2023.1258823

AND CLINICAL DATA SUGGEST NEUROGENIC PATHWAYS TRIGGERED BY IL31RA/OSMR+ NEURONS INFLUENCE TYPE 2 INFLAMMATION IN MULTIPLE TISSUES



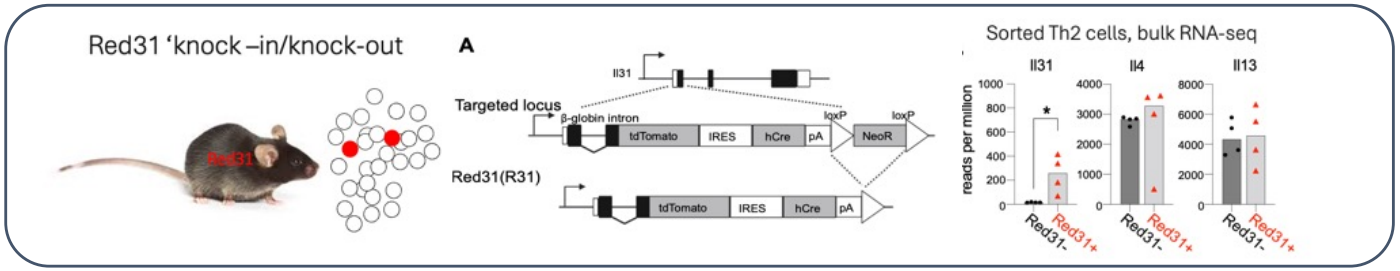
n (%)	Placebo n=91	Nemo 30 mg n=183	Placebo n=96	Nemo 30 mg n=190
Selected adverse events*	9 (9.9)	21 (11.5)	18 (18.9)	32 (17.1)
Newly diagnosed asthma or worsening (w) of asthma (post-adjudication by IAC)				
Asthma	1 (1.1)¶	2 (1.1)¶	2 (2.1)‡¶	6 (3.2)‡¶
Peak expiratory flow decreased	0	3 (1.6)§	-	-
Peripheral edema: limbs, bilateral; facial edema	2 (2.2)	6 (3.3)	1 (1.1)	5 (2.7)
Dermatitis atopic/eczema	0	10 (5.5)	1(1.1)	10 (5.3)
Worsening of AD	-	-	-	-
Neurodermatitis (worsening of PN)**	10 (11.0)	7 (3.8)	19 (20.0)	18 (9.6)



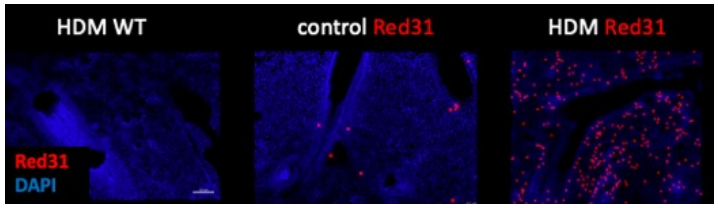
Sofen et al (2023) *eClinicalMedicine* doi: 10.1016/j.eclinm.2023.101826
Mack, M et al. https://doi.org/10.3389/fnmol.2023.1258823

WE TURNED OUR ATTENTION TO IDENTIFICATION OF ENDOGENOUS PATHWAYS THAT REGULATE IL-31 PRODUCTION IN T CELLS

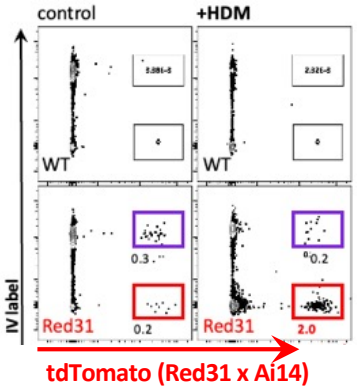
OUR NEW *IL31* TRANSCRIPTIONAL REPORTER (*RED31)* TRANSGENIC MOUSE EXPOSES TYPE 2 STIMULUS-DEPENDENT *IL31* TRANSCRIPTIONAL INDUCTION IN LUNG TYPE 2 MODELS



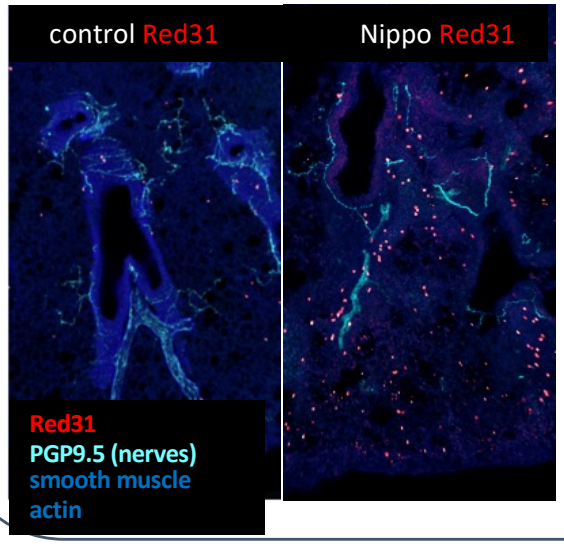
I. house dust mite asthma model



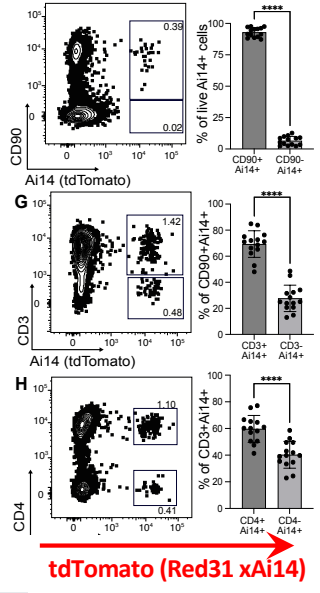
gated on lung CD4 T cells



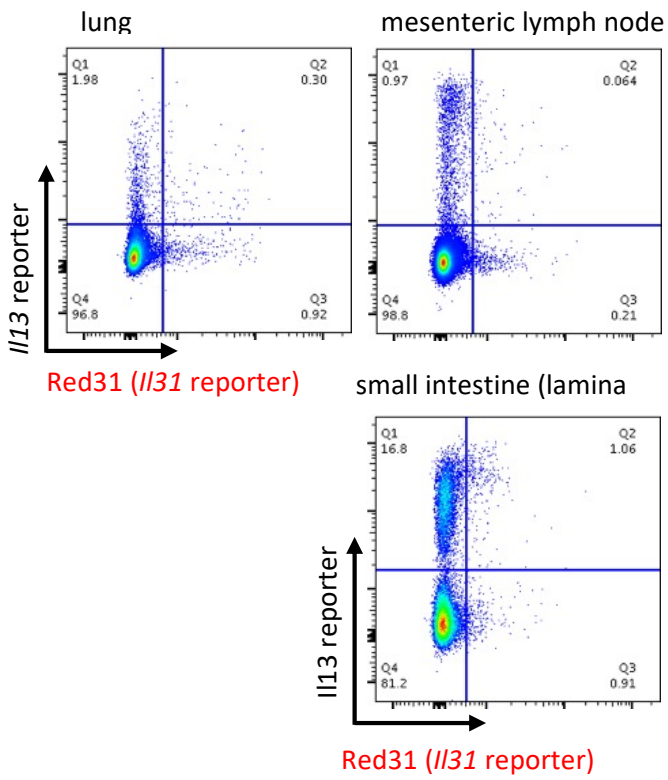
II. Nippostrongylus *brasiliensis helminth infection model*



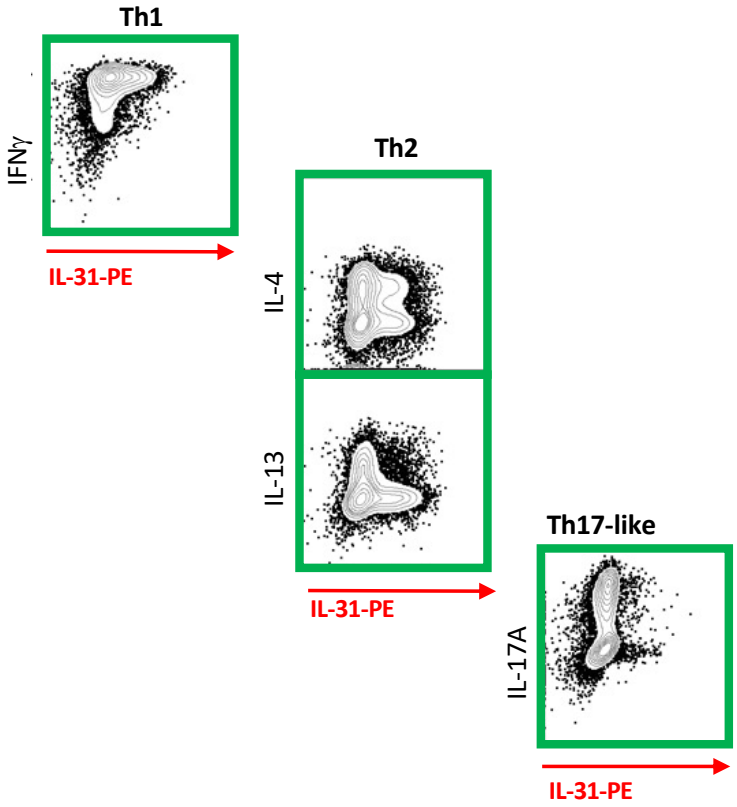
gated on lung CD45+ cells



INFLAMED MOUSE TISSUES & IN VITRO DIFFERENTIATED T CELL SUBSETS REVEAL AN IRREGULAR RELATIONSHIP BETWEEN EXPRESSION OF IL-31 AND EFFECTOR CYTOKINES



Nippostrongylus brasiliensis, mouse organs d5 post-infection



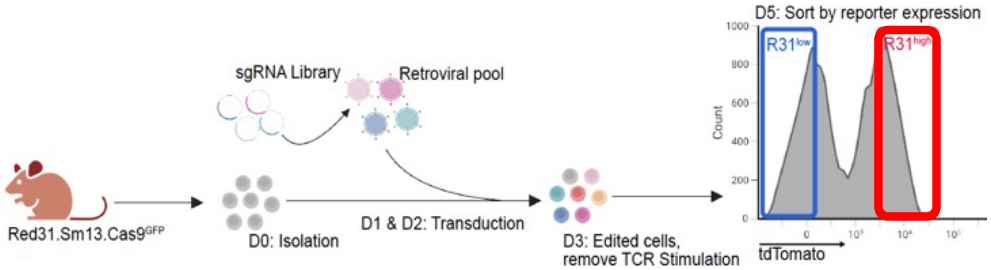
in vitro –differentiated CD4 T cell subsets

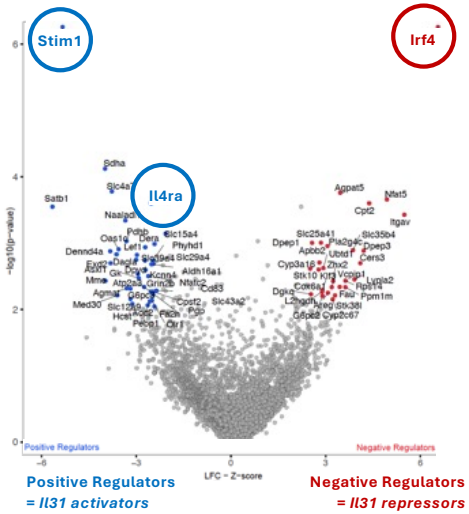
THERE'S GROWING INTEREST IN BETTER UNDERSTANDING HOW IL-31 FITS INTO THE TYPE 2 INFLAMMATORY DISEASE SPACE

AND HOW IT IS SIMILAR TO/DIFFERENT FROM CANONICAL TYPE 2 CYTOKINES

IN HOPES OF OPTIMIZING IL-31/IL31RA REGULATION IN TYPE 2 DISEASES

TO IDENTIFY CANDIDATE *IL31* REGULATORS, WE PERFORMED A QUARTER-GENOME CD4 T CELL CRISPR SCREEN IN TH2 CONDITIONS





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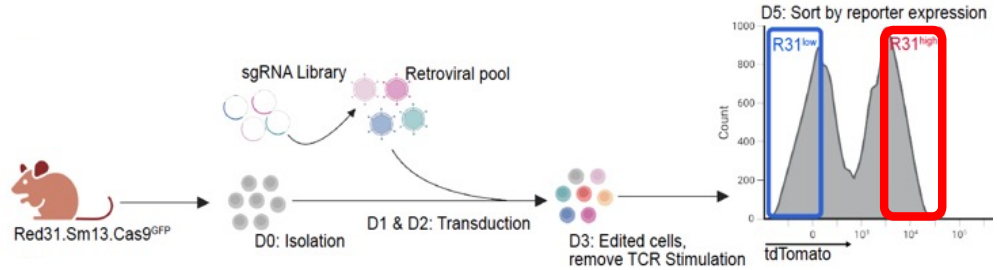
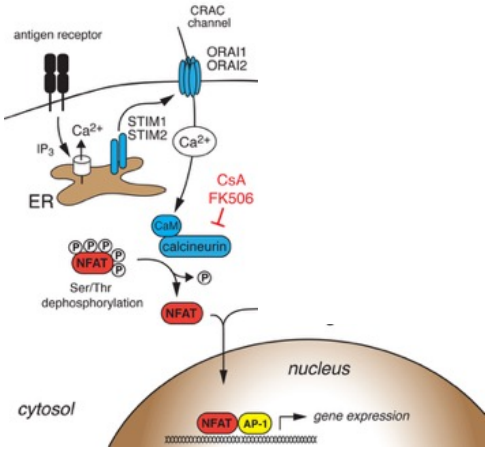
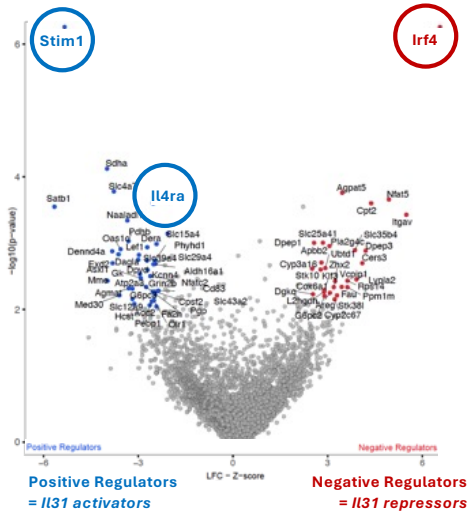
Il31 ACTIVATORS INCLUDE FACTORS IN T CELL ACTIVATION, TH2 DIFFERENTIATION







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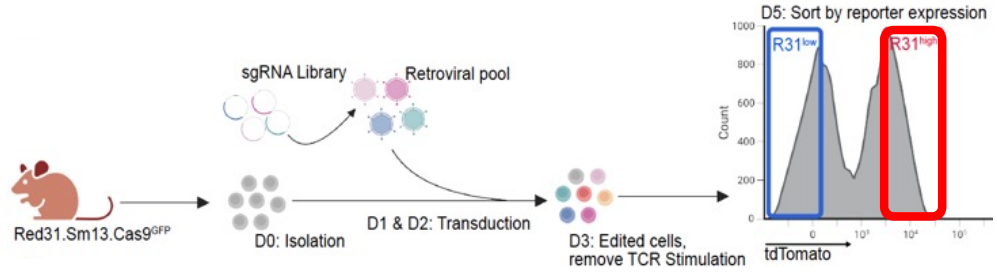
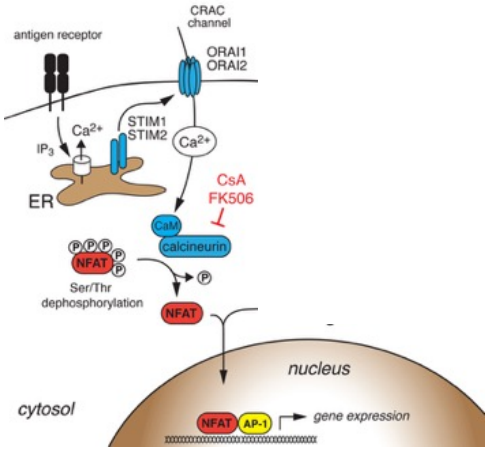
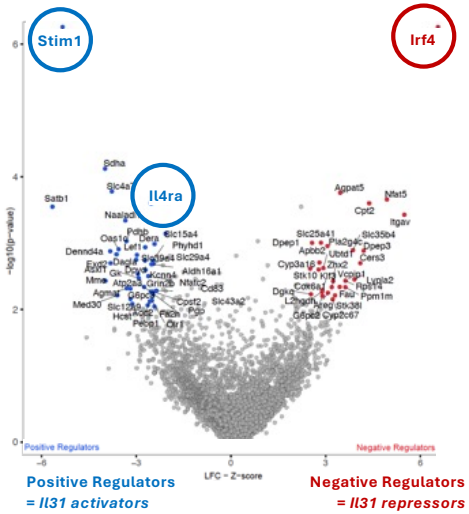
IL31 ACTIVATORS INCLUDE FACTORS IN T CELL ACTIVATION: STIM1/CALCIUM SIGNALING

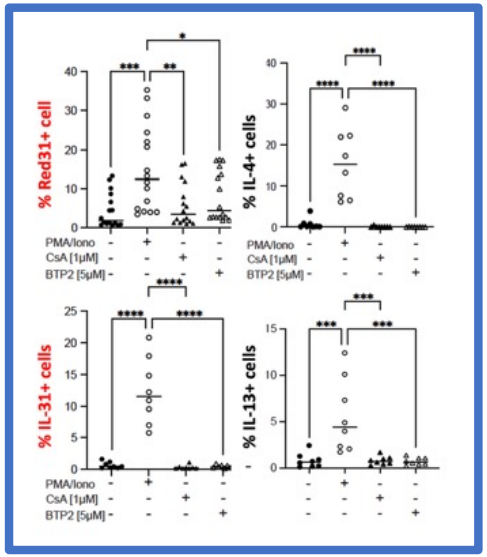






Positive Regulators
= Il31 activators

Negative Regulators
= Il31 repressors



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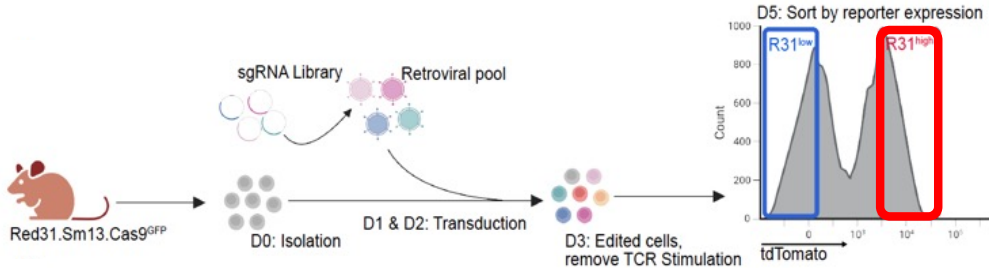
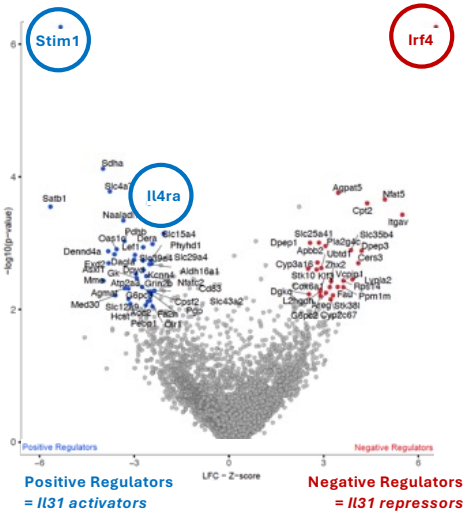
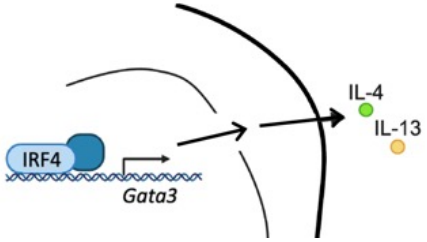
THE STRONGEST *IL31* REPRESSOR CANDIDATE WAS *IRF4*, A PRO- TH2 TRANSCRIPTION FACTOR







<u>Transcriptional targets of *IRF4:*</u>

- Th2 master transcription factor GATA3 (direct)
- **Th2 cytokine locus (hu chr 5, m chr 11) is a**
  direct target of IRF4
  indirect target via IRF4 >> GATA3

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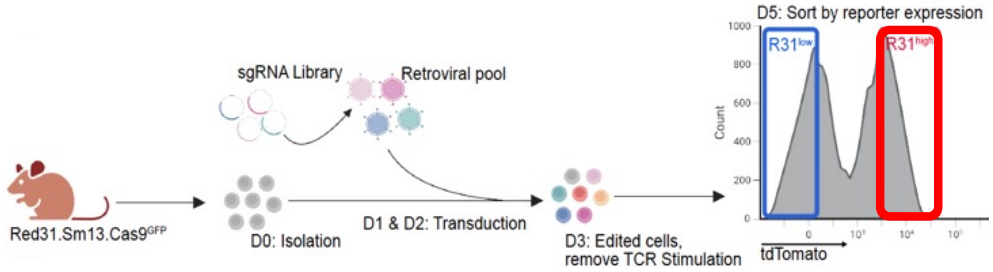
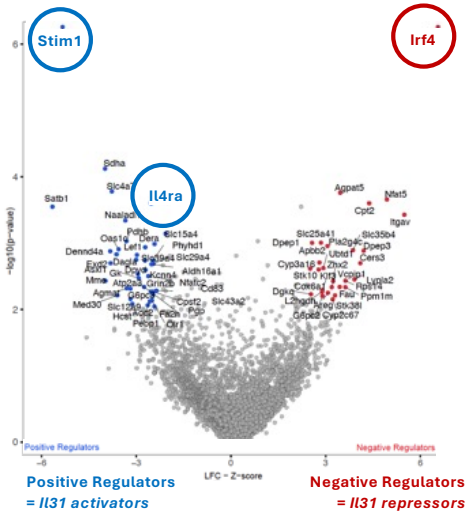
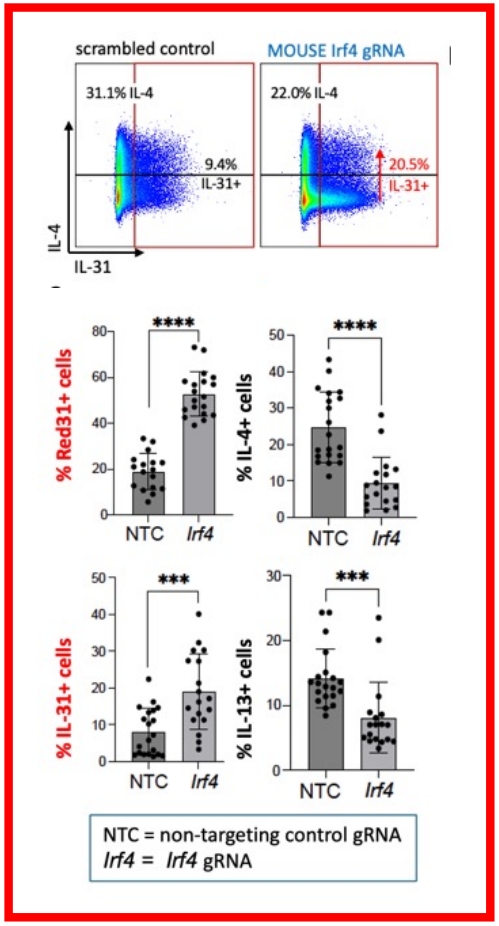
CRISPR-EDITING *IRF4*, A PRO-TH2 TRANSCRIPTION FACTOR* LED TO ↑IL-4/IL-13 & ↓IL-31!

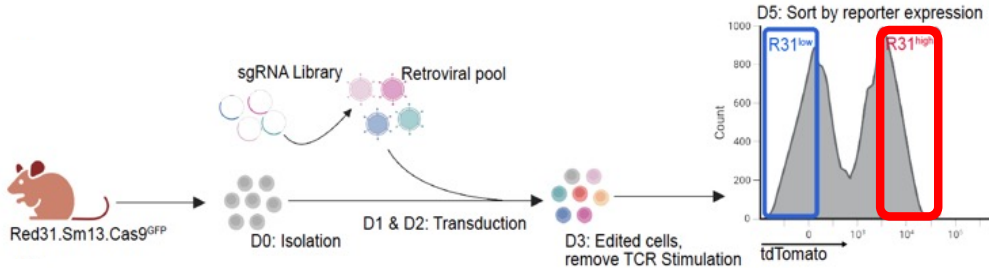


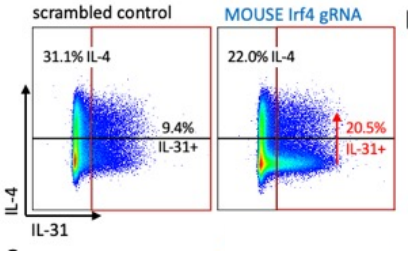


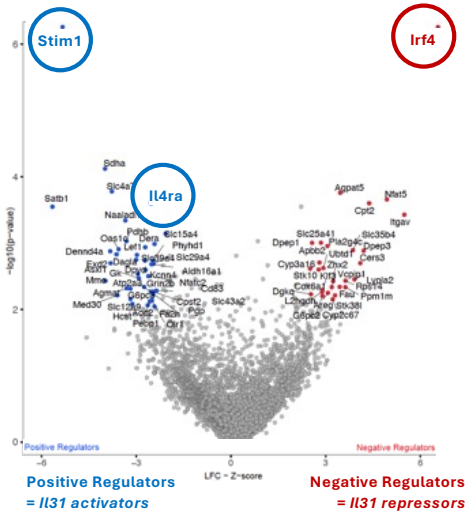


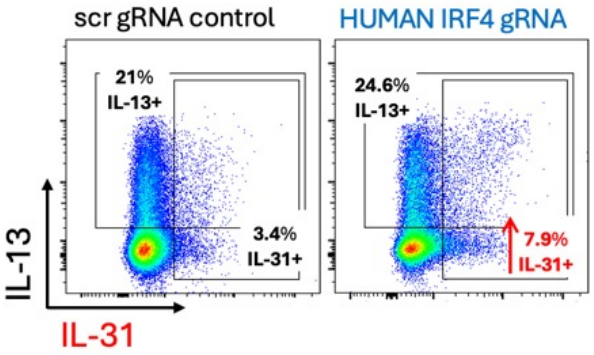
*Lohoff M … Mak TW. *Proc Natl Acad Sci USA*. 2002; 99(18):11808-12. doi: 10.1073/pnas.182425099.

IRF4-DEPENDENT REPRESSION OF *IL31* IS CONSERVED IN MOUSE/HUMAN CD4 T CELLS



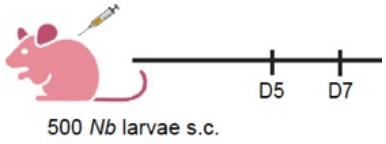






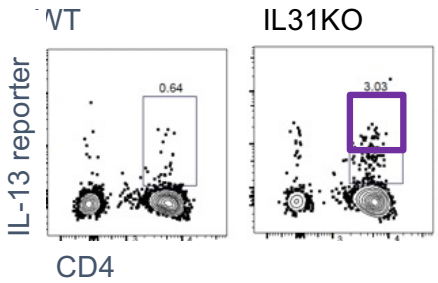
*Lohoff M … Mak TW. *Proc Natl Acad Sci USA*. 2002; 99(18):11808-12. doi: 10.1073/pnas.182425099.

TO EXAMINE **FUNCTIONAL CONSEQUENCES OF IRF4-DEPENDENT *IL31* REPRESSION**,
WE USED THE *Nb* LUNG-GUT PARASITE MODEL,
WHERE THE *Nb* RESPONSE INVOLVES INCREASED LYMPHOID CELL IL-31 EXPRESSION, AND
THE IL31KO PHENOTYPE IS INCREASED INTESTINAL TYPE 2 INFLAMMATION:

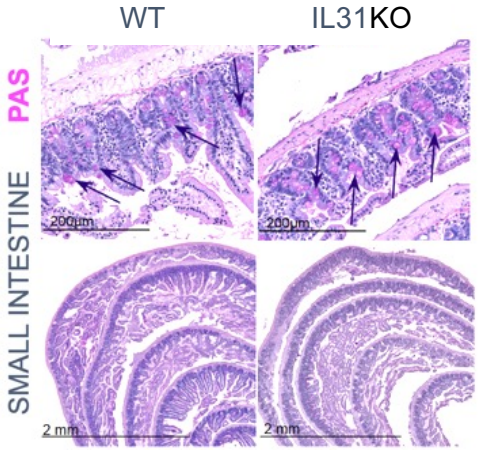


GUT

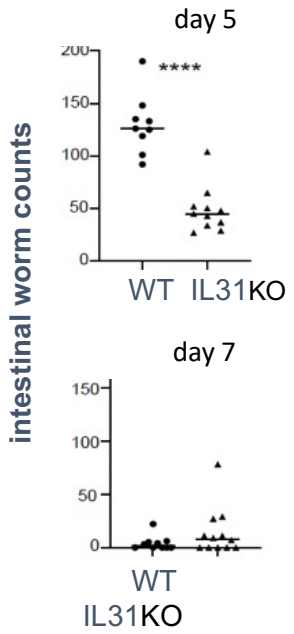
more **IL-13+ (Th2) T cells**



more **mucus+ goblet cells**



faster **worm clearance**
(stronger Th2 response)



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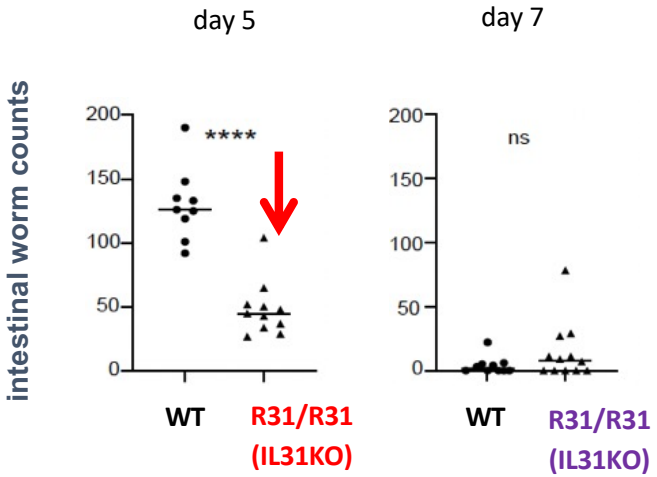
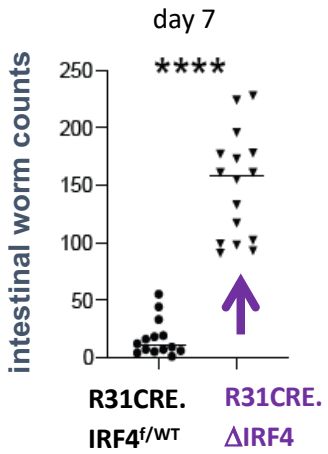
IN CONTRAST, ***IRF4* DELETION *ONLY IN IL31(RED31)+ CELLS*** *DELAYED/IMPAIRED WORM CLEARANCE, INDICATING IMPAIRED SYSTEMIC TYPE 2 INFLAMMATORY RESPONSES*

R31/R31 (IL31KO)
faster **worm clearance**
(stronger Th2 response)

R31CRE.ΔIRF4
slower **worm clearance**
(insufficient Th2 response)





WHAT DOES THIS MEAN? IRF4 ACTIVITY (TURNING ON GATA3, IL4, IL13) SPECIFICALLY IN *R31(IL31)+ CELLS SETS THE MAGNITUDE OF SYSTEMIC TYPE 2 RESPONSE*

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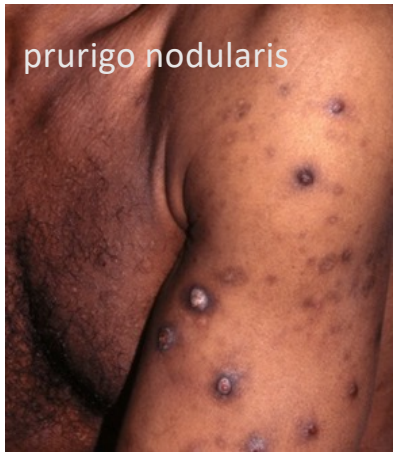
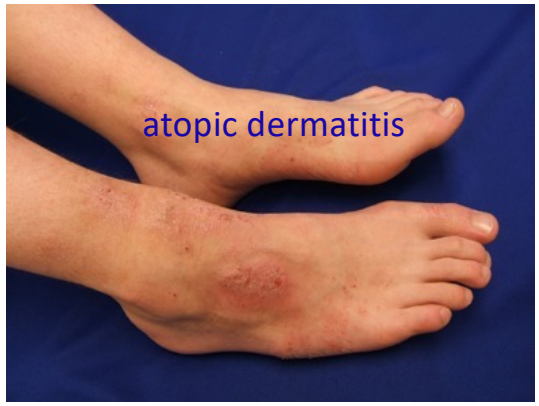
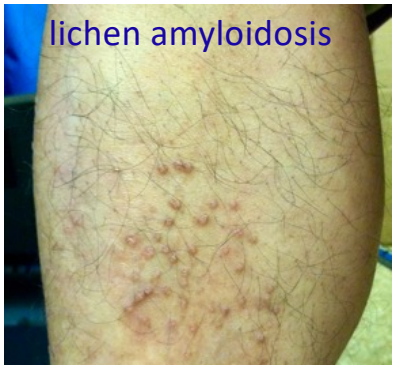
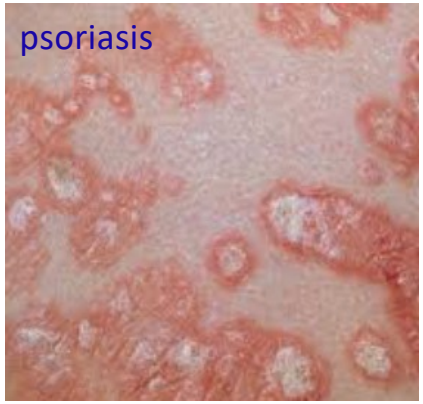
WHAT DOES THIS MEAN FOR SKIN CONDITIONS WITH ELEVATED IL-31?







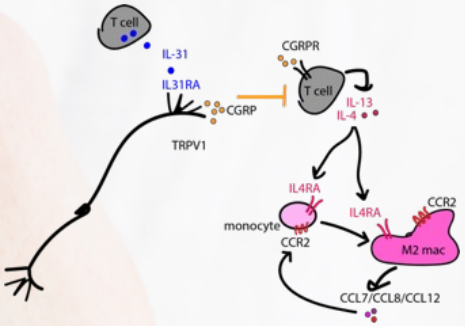


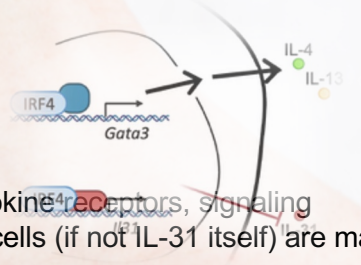
WHAT DOES THIS MEAN FOR TYPE 2 SKIN CONDITIONS WITH ELEVATED IL-31?

Atopic dermatitis and other type 2 diseases of skin and lung involve self-perpetuating inflammatory loops and counterregulatory mechanisms.

Many individual cytokine genes, especially those encoded in the type 2 locus control region (Il4, Il5, IL13) are regulated by interconnected circuits or by common transcription factors; others are more likely to be turned on/off by completely different inputs

For example, the composition of IRF4 heterodimers determines its set of transcription target genes; the combination of available TFs available to bind target gene loci can result in different expression programs. Local and genome-wide changes in chromatin accessibility add another layer of complexity.





Effective biologics and small molecules that target cytokine circuitry have already highlighted key nodes (cells, cytokine receptors, signaling cascades) that dominantly impact disease outcomes. Based on our recent work, we propose that IL-31 producing cells (if not IL-31 itself) are master regulators of type 2 disease outcomes.

CONTRIBUTORS AND ACKNOWLEDGMENTS

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