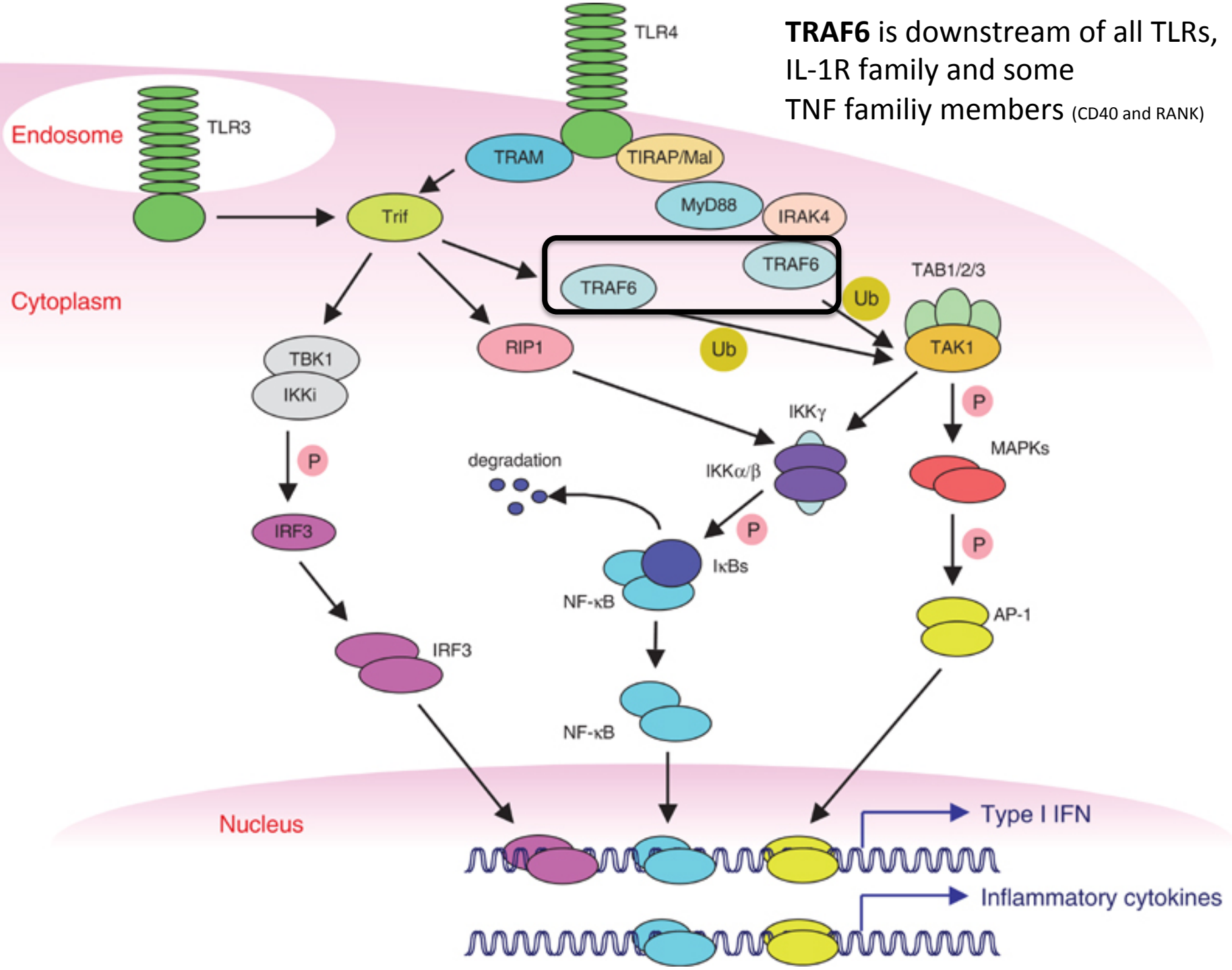
Immunity
Article

Dendritic Cell Expression of the Signaling Molecule TRAF6 Is Critical for Gut Microbiota-Dependent Immune Tolerance

Daehee Han,[1,7] Matthew C. Walsh,[1] Pedro J. Cejas,[1] Nicholas N. Dang,[1] Youngmi F. Kim,[1] Jihyun Kim,[1] Laetitia Charrier-Hisamuddin,[2] Lillian Chau,[2] Qin Zhang,[3] Kyle Bittinger,[3] Frederic D. Bushman,[3] Laurence A. Turka,[6] Hao Shen,[3] Boris Reizis,[5] Anthony L. DeFranco,[4] Gary D. Wu,[2] and Yongwon Choi[1,7,*]

[1]Department of Pathology and Laboratory Medicine
[2]Division of Gastroenterology
[3]Department of Microbiology
University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA
[4]Department of Microbiology and Immunology, University of California, San Francisco, San Francisco, CA 94143, USA
[5]Department of Microbiology and Immunology, Columbia University Medical Center, New York, NY 10032, USA
[6]Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02215, USA
[7]Division of Integrative Biosciences and Biotechnology, Pohang University of Science and Technology, Pohang, Republic of Korea
*Correspondence: ychoi3@mail.med.upenn.edu
http://dx.doi.org/10.1016/j.immuni.2013.05.012

TRAF6 is downstream of all TLRs, IL-1R family and some TNF familiy members (CD40 and RANK)



TRAF6$^{-/-}$ (deficient for TRAF6 in all cells)

TRAF6-/- die perinatally (max. survival day 15)

TRAF6-/- DCs (derived from complete KO)

- can prime T cells less well to produce IFNγ
- do not fully mature upon TLR/IL1R stimulation

(Kobayashi 2003)

Fetal liver-chimeras with TRAF6-/- cells → organ infiltration of Th2 cells
(Chiffoleau, 2003)

→ View: TRAF6-deficieny DCs have a maturation defect and therefore have a reduced T cell-priming capacity

Despite this defective activation phenotype, TRAF6$^{-/-}$ DC are linked to an spontaneous development of Th2 inflammation in the intestine

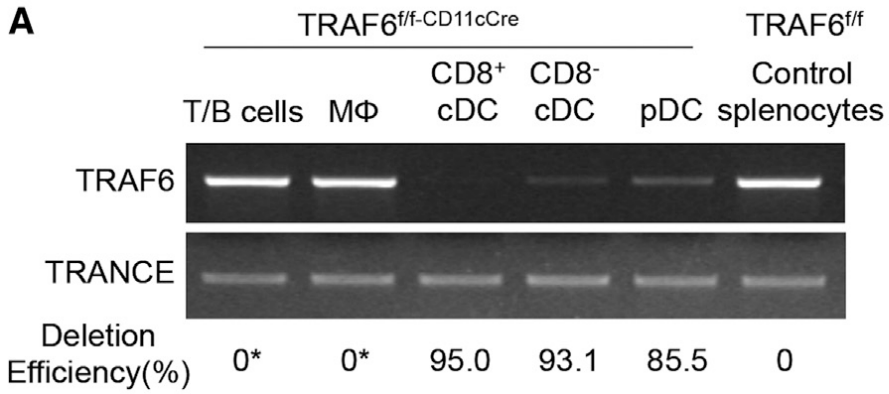
"DC-specific" delection of TRAF6 → CD11cCreTRAF6f$^{l/fl}$

CD11c not only in DCs

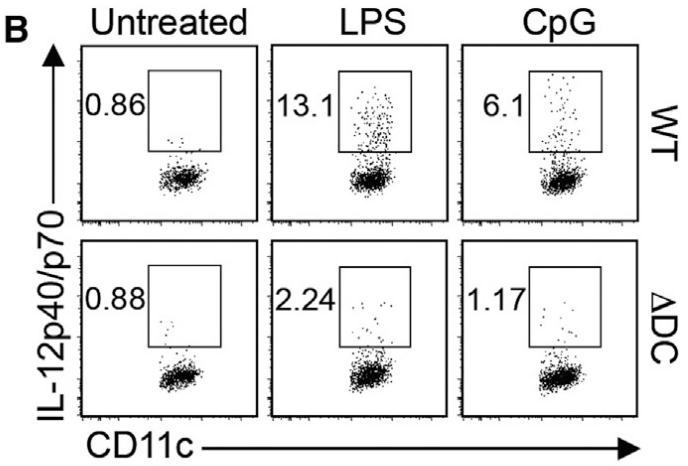
Specificity not convincingly shown!

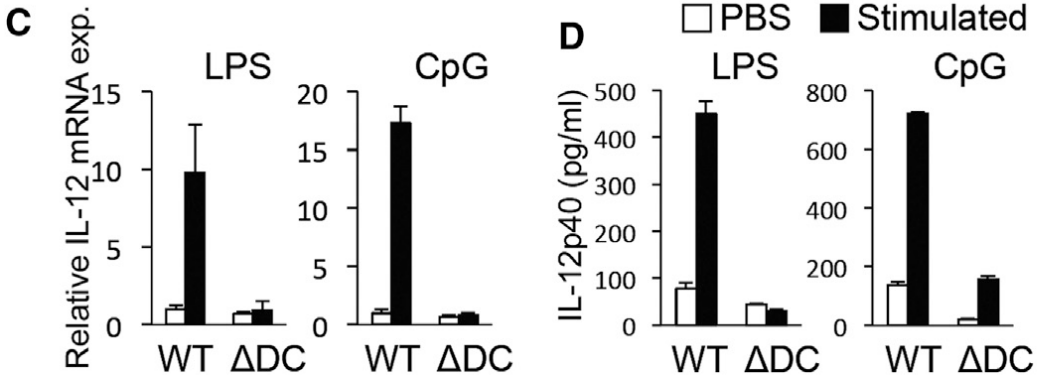
"Specific deletion was further observed in DCs isolated from other tissues as well (not shown)"

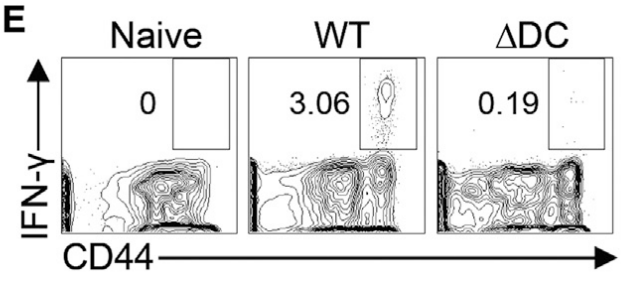
sorted splenocytes, no sorting markers indicated!



FACS: In vitro stimulation (PMA/Ionomycin of splenocytes)

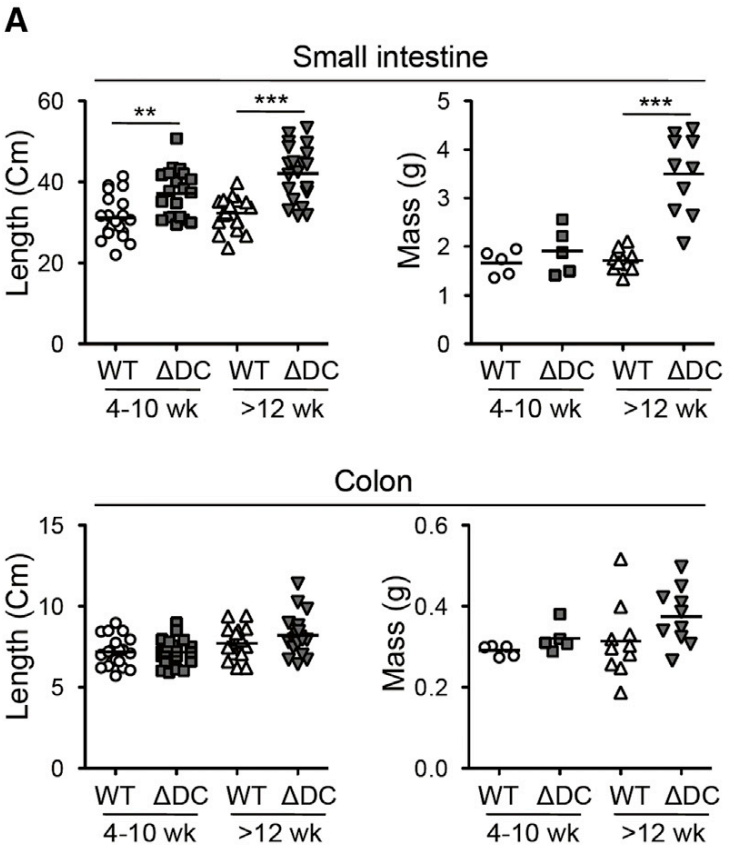


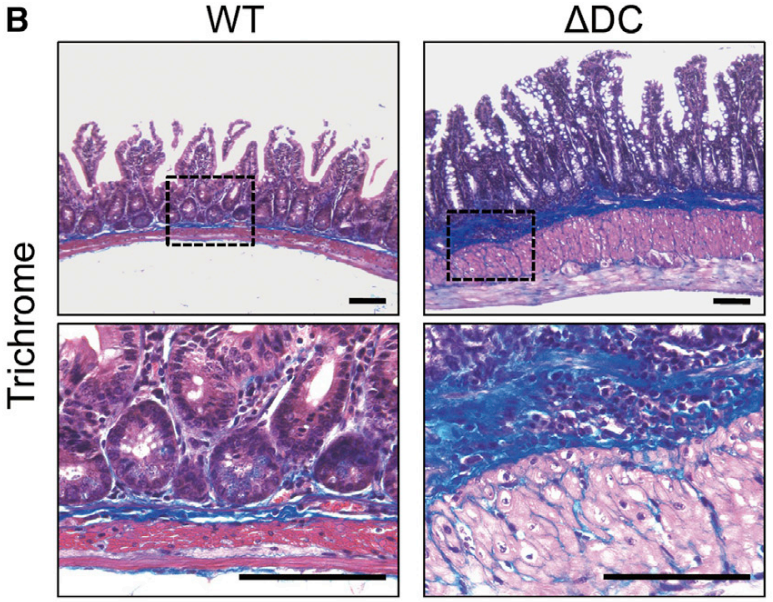


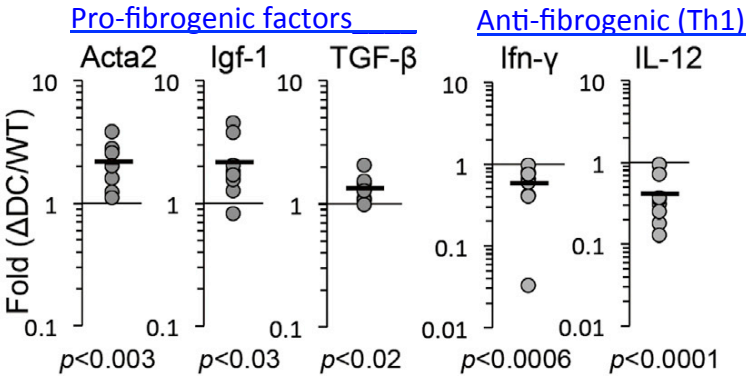


qPCR: In vitro stimulation (PMA/Ionomycin of splenocytes)

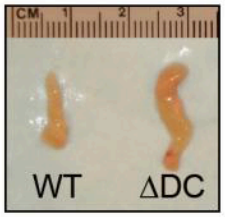
LCMV; spleen d7 post infection, CD4+ gate







12 weeks

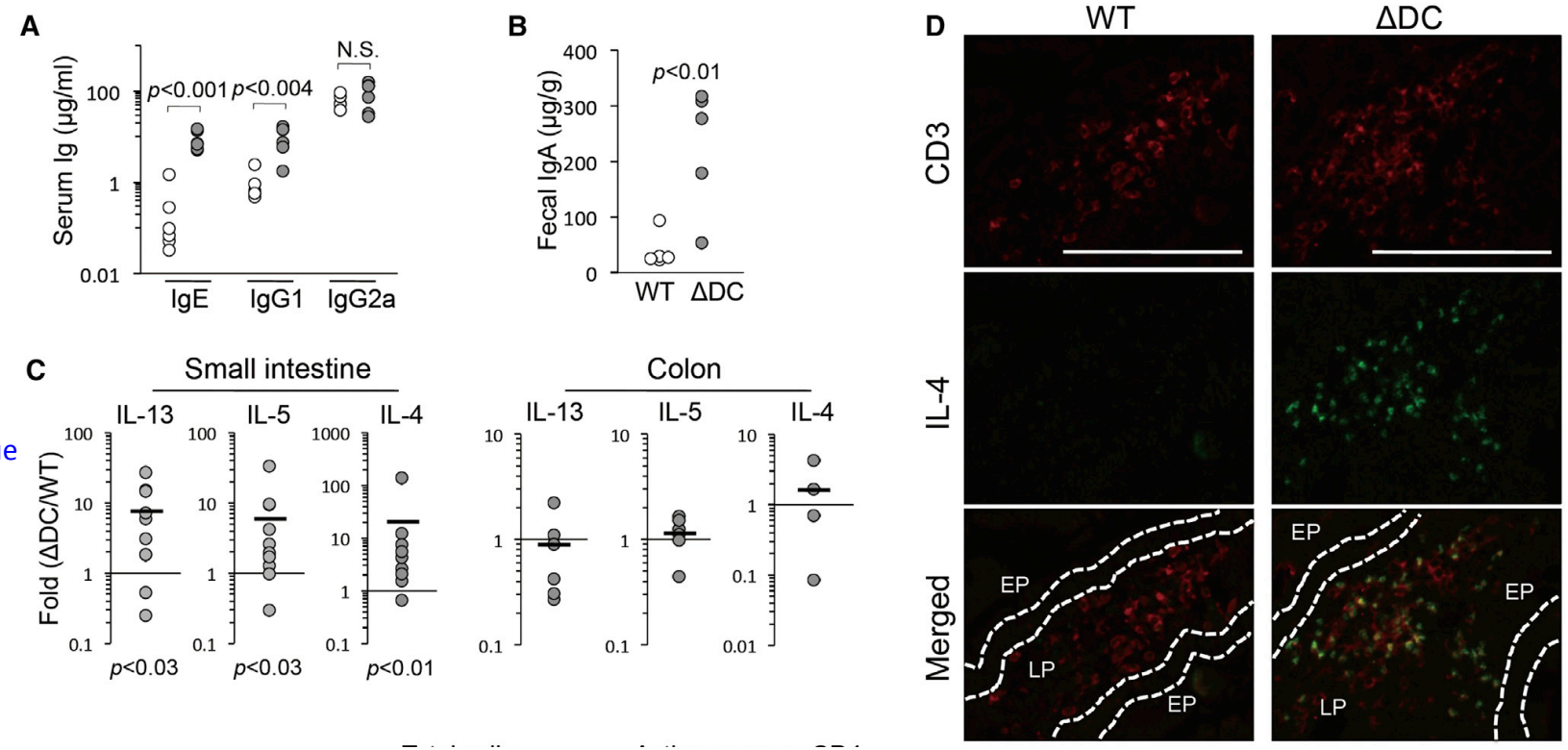


Th2 inflammation:

- Tissue length and mass increases
- Enteritis
- Thickening of smooth muscle layer
- Hypertrophy of intestinal crypts
- Goblet and Paneth cells increase
- Fibrosis

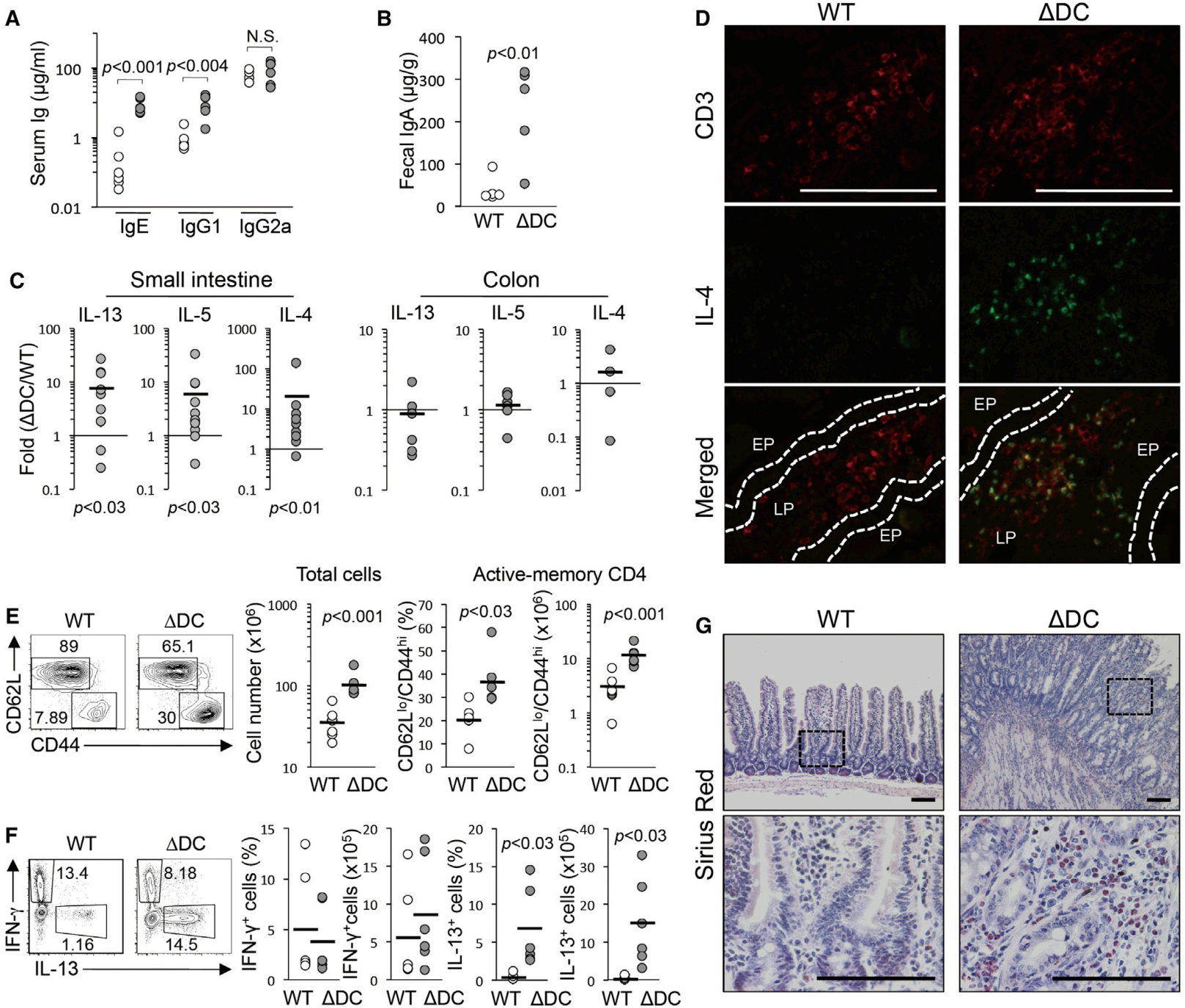
Th2 inflammation is conferred by T cells

Th2-cytokines co-localise with CD3
→ Source are T cells

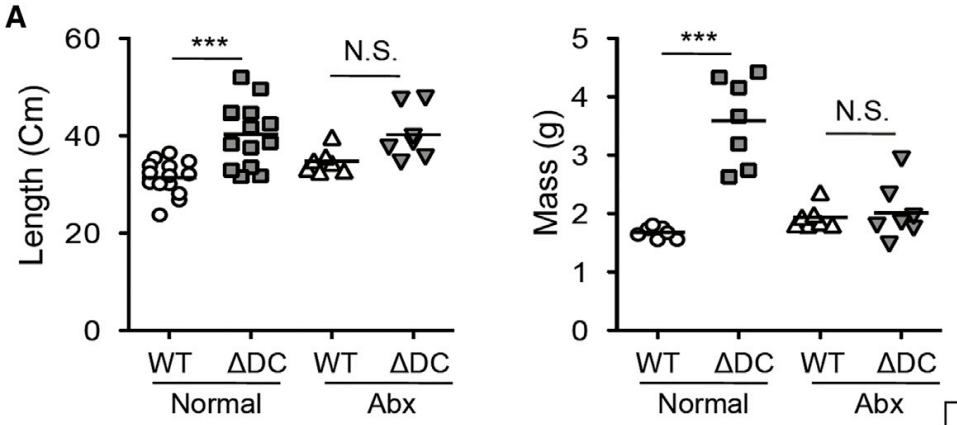


At 8 weeks,
Whole tissue

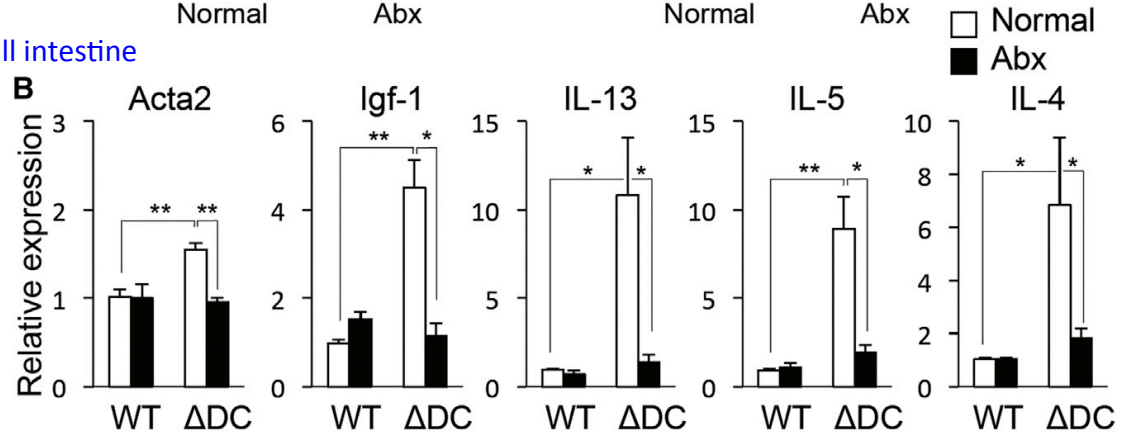
Suppl: No inflammation seen in TRAF6ΔDC x SCID


A
Serum Ig (μg/ml)
100
1
0.01
p<0.001
p<0.004
N.S.
IgE
IgG1
IgG2a
B
Fecal IgA (μg/g)
400
300
200
100
0
p<0.01
WT
ΔDC
C
Small intestine
Colon
IL-13
IL-5
IL-4
Fold (ΔDC/WT)
p<0.03
p<0.03
p<0.01
D
WT
ΔDC
CD3
IL-4
Merged
EP
LP
E
WT
ΔDC
89
7.89
65.1
30
CD62L
CD44
Total cells
Active-memory CD4
Cell number (x10⁶)
CD62L^lo/CD44^hi (%)
CD62L^lo/CD44^hi (x10⁶)
p<0.001
p<0.03
p<0.001
F
WT
ΔDC
13.4
1.16
8.18
14.5
IFN-γ
IL-13
IFN-γ⁺ cells (%)
IFN-γ⁺cells (x10⁵)
IL-13⁺ cells (%)
IL-13⁺ cells (x10⁵)
p<0.03
p<0.03
G
WT
ΔDC
Sirius Red

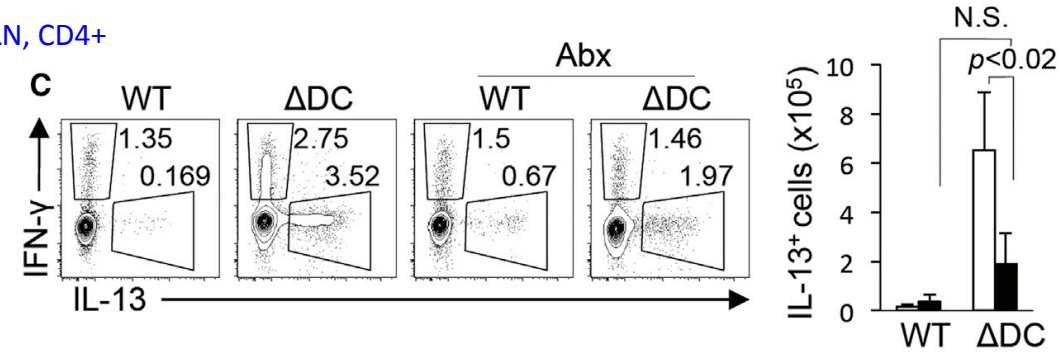
Antibiotic treatment prevents small intestinal inflammation

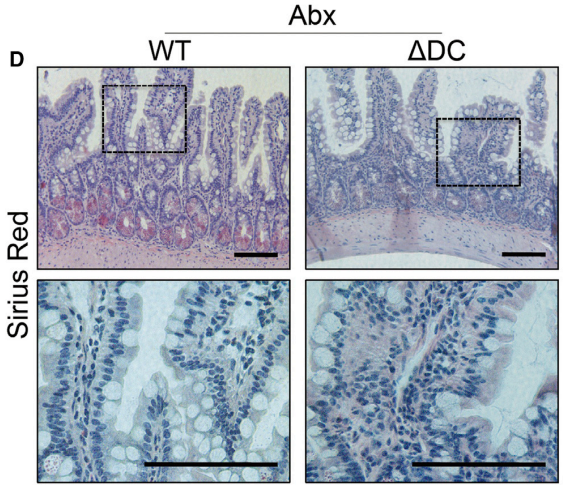


Small intestine



MLN, CD4+



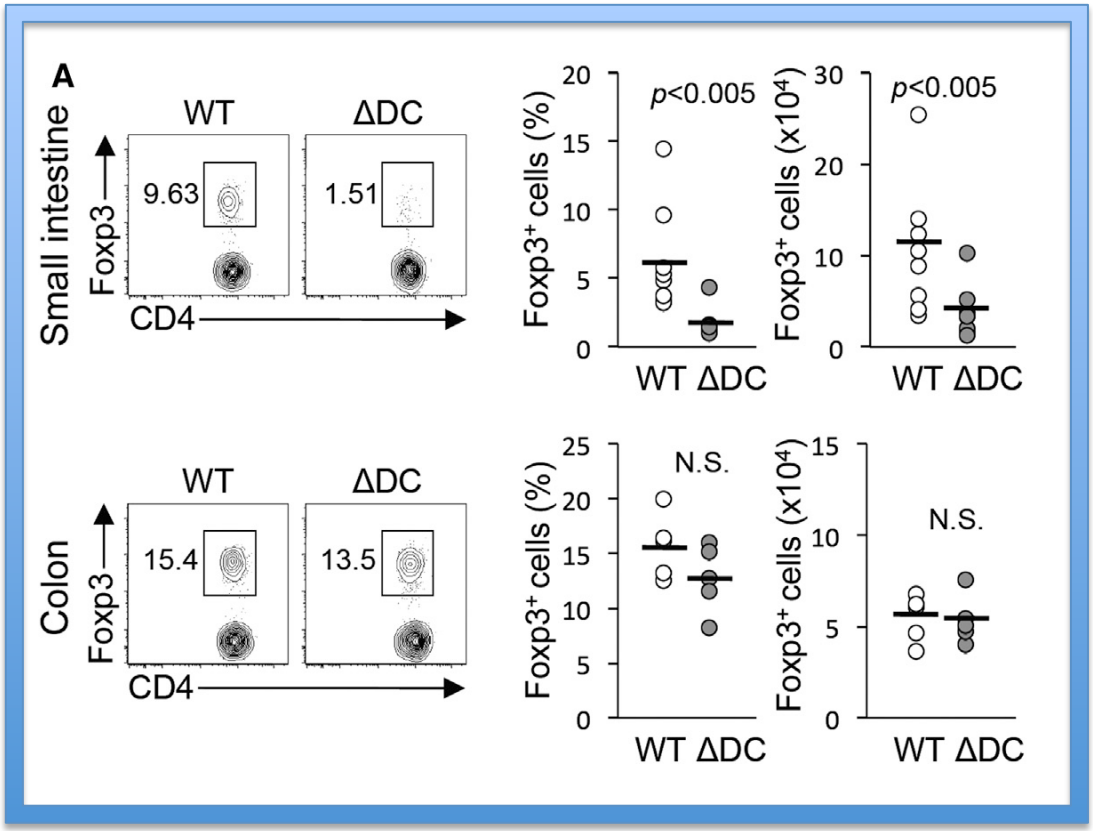


Treatment:

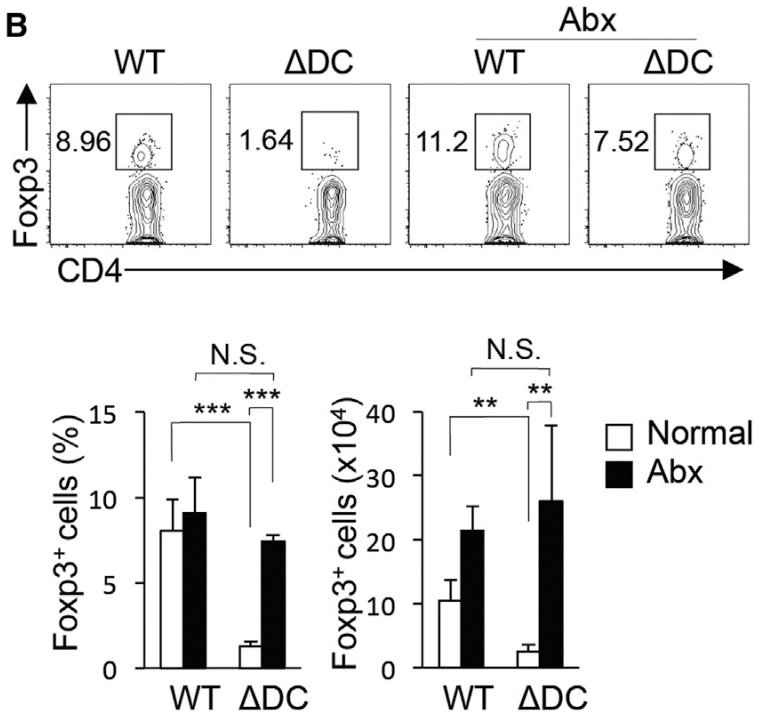
1g/L Ampicillin
1g/L Neomycin
0.5g/L Vancomycin
1g/L Metronidazol

In drinking water ad libitum for 6 weeks
Starting at 14 weeks of life

Microbiota-dependent reduction of Tregs in the small intestine, but not the colon in TRAF6ΔDC



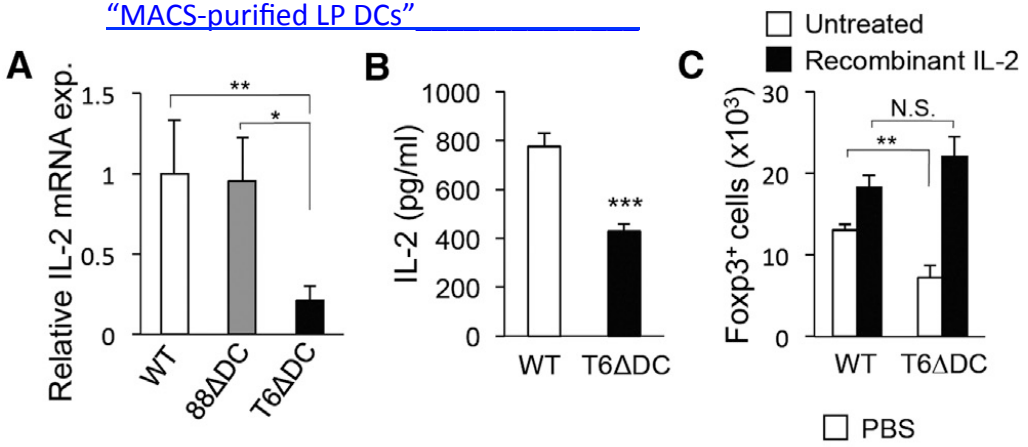
Abx from week 4 of life, for 2 weeks



In TRAF6DDC mice Abx treament restores the Treg (FoxP3+ cells) number

Possible mechanism of diminished Tregs counts: Reduces IL-2 producing capacity of TRAF6ΔDC

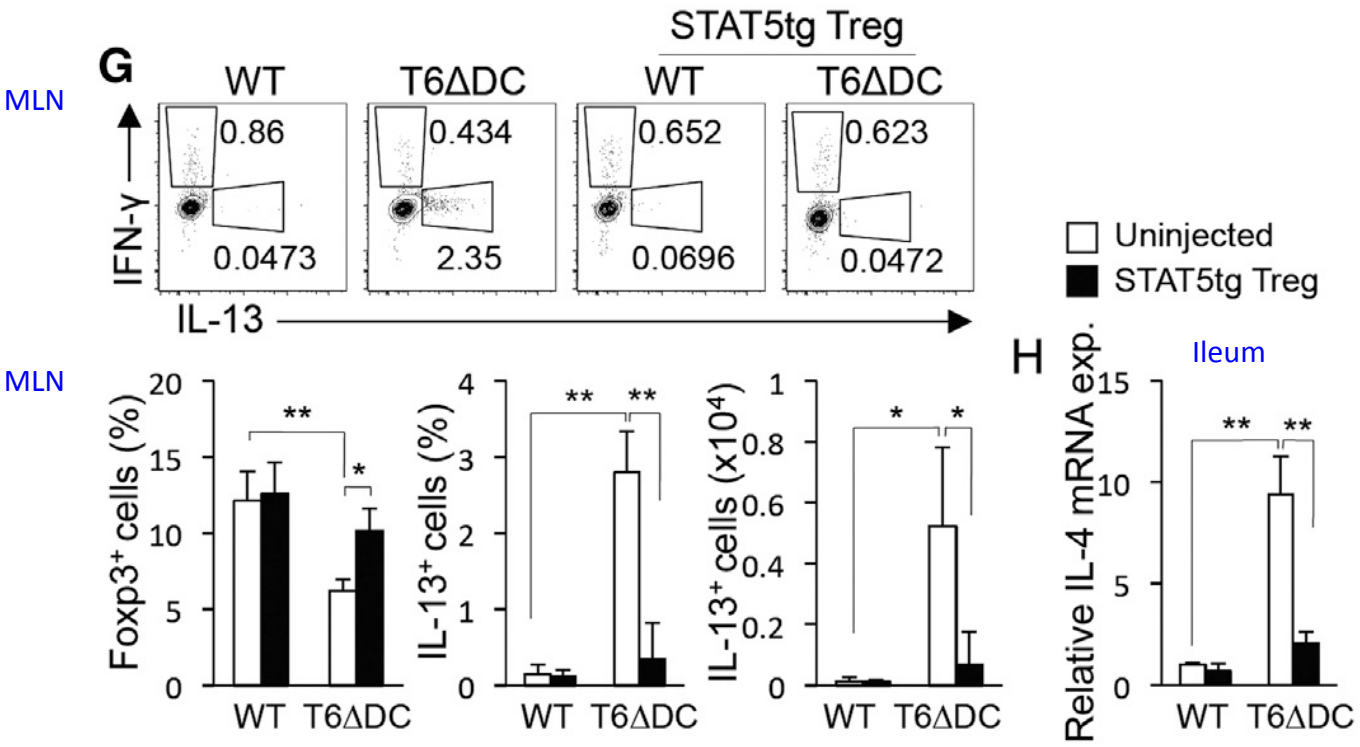
"MACS-purified LP DCs"



In vitro conversion to Tregs
Co-culture of OT-II with DCs + Ova
4 days

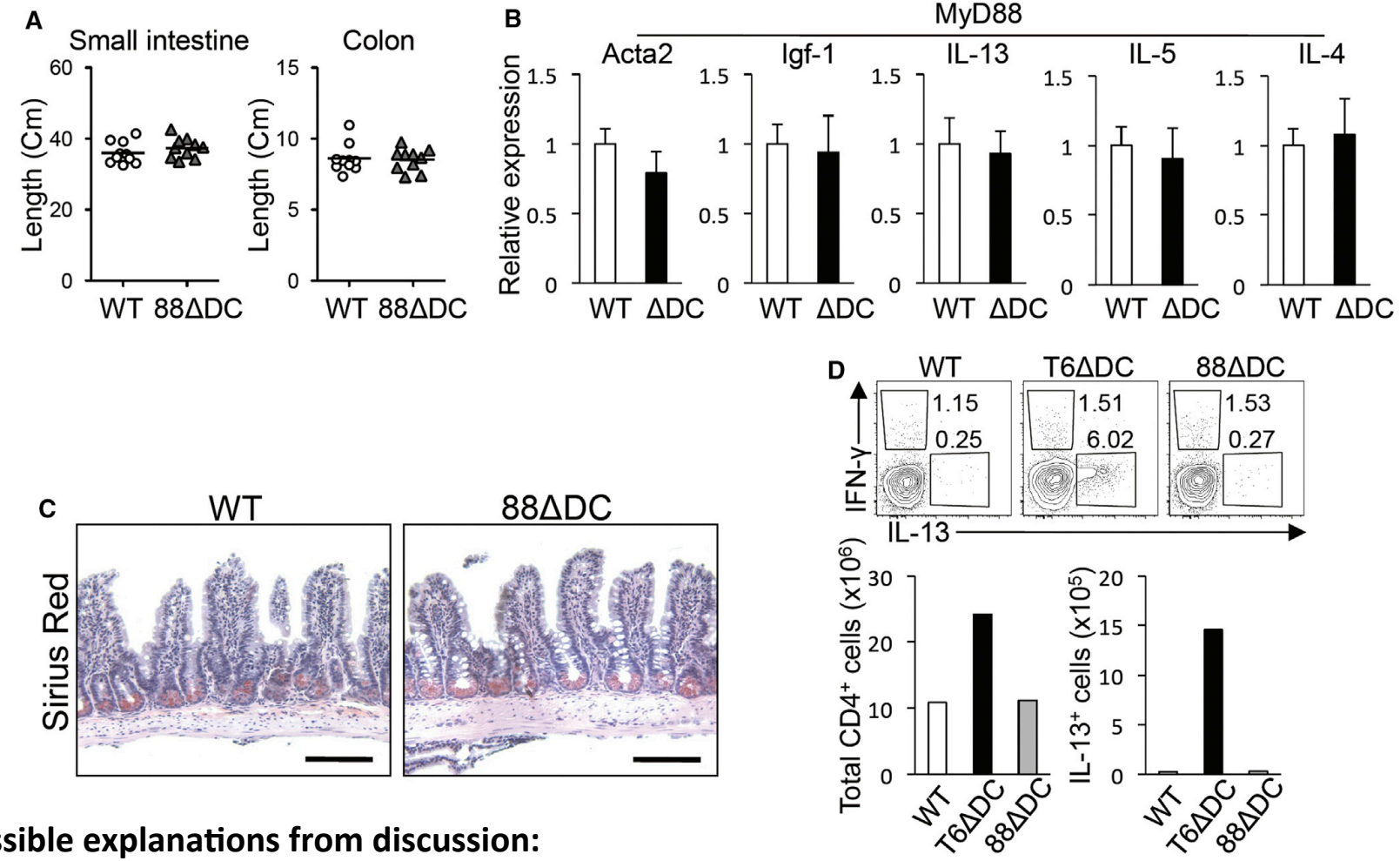
Transferred Tregs can prevent induction of Th2 inflammation

Sorted Tregs from STAT5tg FoxP3-GFP+ tranferred into T6ΔDC or WT mice
4 weeks post transfer



MyD88ΔDC mice do not phenocopy aberrant immune homeostasis of TRAF6ΔDC mice

Woundn't we expect that if Abx-treated mice do not show inflammation?



Possible explanations from discussion:

1. TLRs activate a MyD88-independent, but TRAF6-dependent tolerogenic pathway
2. Microbiota utilise PRR-independent pathways, e.g. ATP (P2X) to act on DCs
3. TLRs stimulate non-DC cells to produce factors that act on DCs in a MyD88-independent, TRAF6-dependent fashion (e.g. TGF-b, CD40L, RANKL, IL-25)

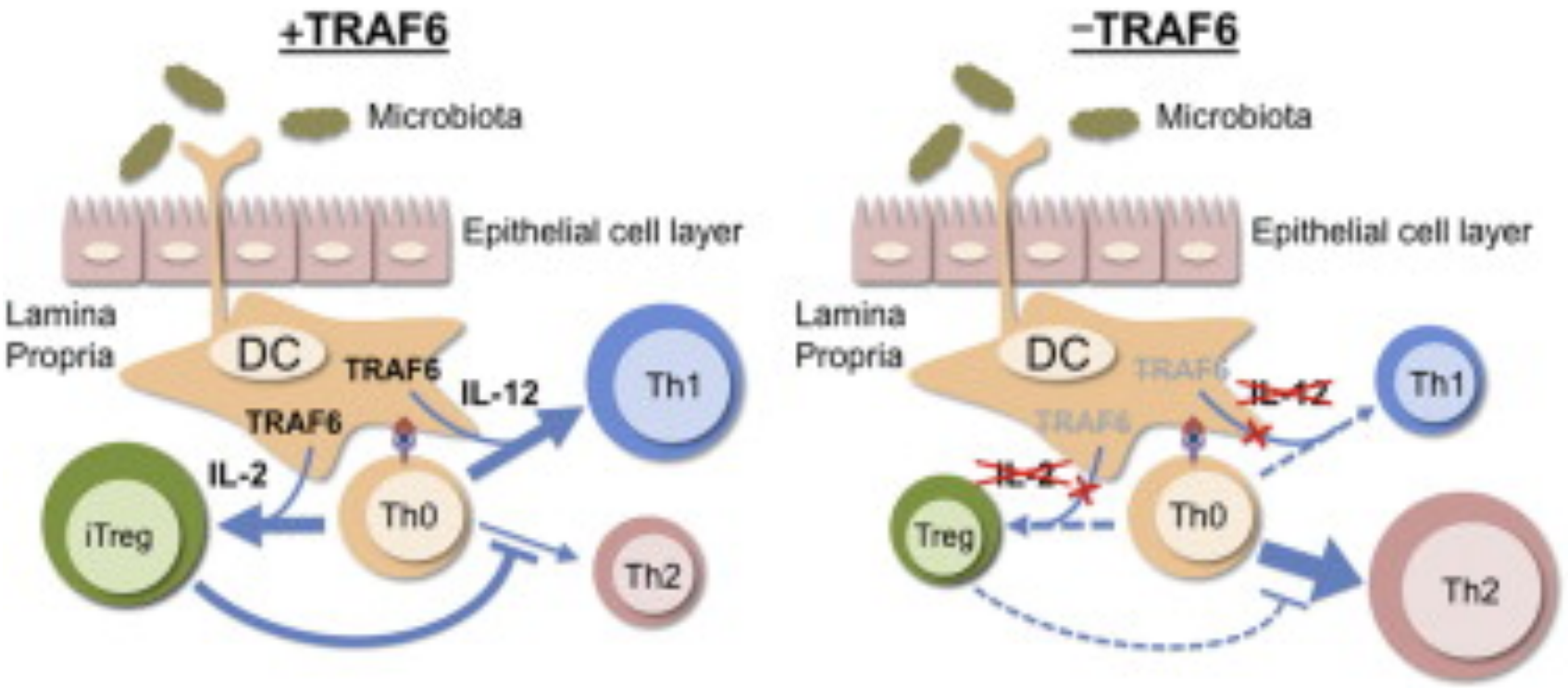
Summary

Dendritic cell (DC)-expressed TRAF6 is critical for small intestine immune tolerance

Spontaneous gut Th2 cell responses develop in the absence of DC-expressed TRAF6

Gut microbiota trigger small intestine autoimmunity absent DC-expressed TRAF6

DC-expressed TRAF6 controls IL-2-associated iTreg cell induction in small intestine



Sciencexpress

The Microbial Metabolites, Short-Chain Fatty Acids, Regulate Colonic T_{reg} Cell Homeostasis

Patrick M. Smith,[1] Michael R. Howitt,[1] Nicolai Panikov,[1] Monia Michaud,[1] Carey Ann Gallini,[1] Mohammad Bohlooly-Y,[5] Jonathan N. Glickman,[6,7] Wendy S. Garrett[1,2,3,4]*

[1]Harvard School of Public Health, Departments of Immunology and Infectious Diseases and Genetics and Complex Diseases, Boston, MA, USA. [2]Harvard Medical School, Department of Medicine, Boston, MA, USA. [3]Dana-Farber Cancer Institute, Department of Medical Oncology, Boston, MA, USA. [4]Broad Institute of Harvard and MIT, Cambridge, MA, USA. [5]AstraZeneca R&D, RAD-Transgenic, Mölndal, Sweden. [6]Harvard Medical School, Department of Pathology, Boston, MA, USA. [7]Miraca Life Sciences, Inc., Newton, MA, USA.

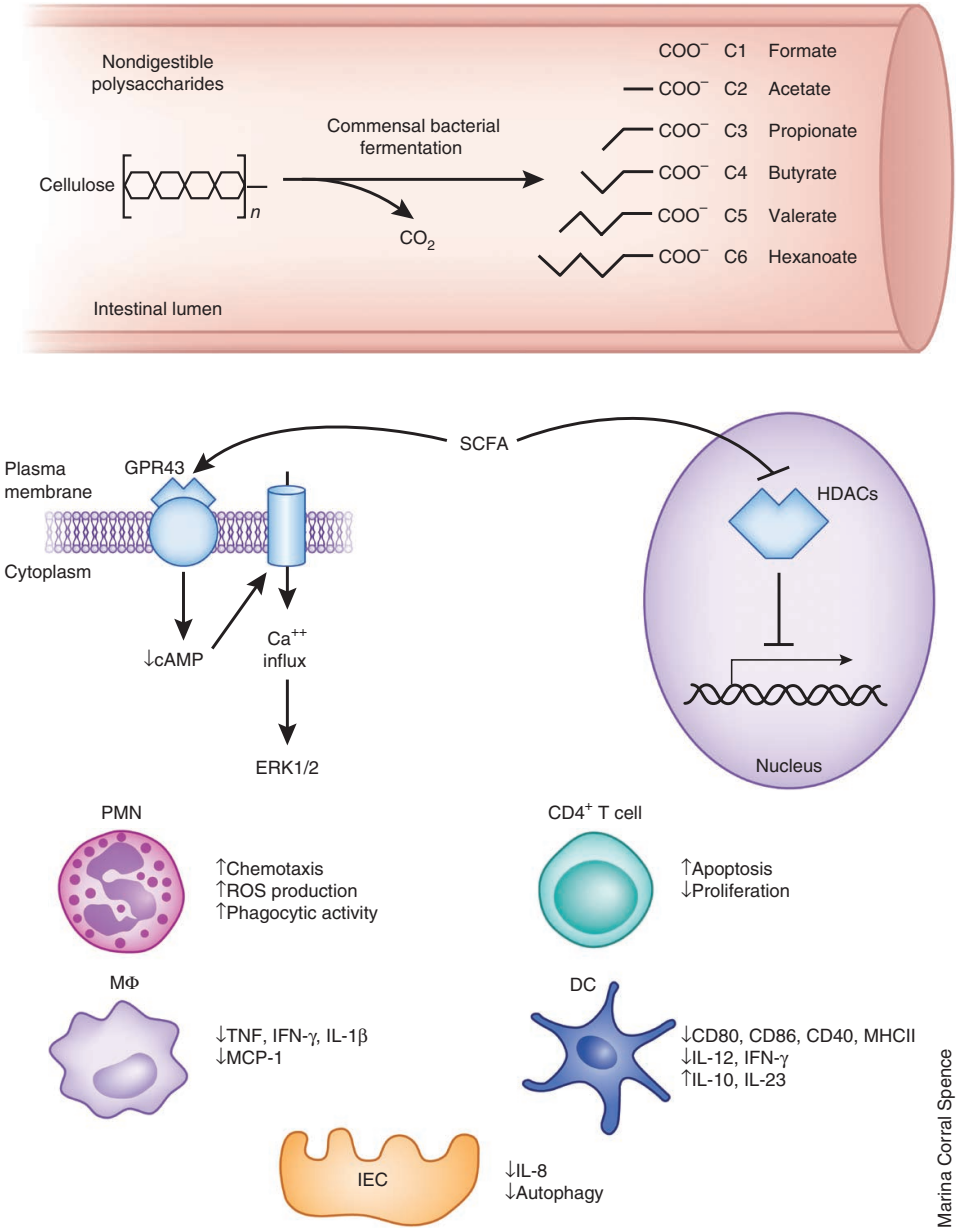
*Corresponding author. E-mail: wgarrett@hsph.harvard.edu

Short-chain fatty acids

- are 1–6 carbons in length
- produced in the colon by bacterial fermentation of plant-derived nondigestible polysaccharides, such as cellulose

Germ-free mice have
- more of the nondigestible plant oligosaccharide raffinose (bacterial fermentation substrate)
- diminished concentrations of SCFA

SCFA can…
…activate GPR43 (free fatty acid receptor 2, FFA2)
…inhibit histone deacetylases
…regulate autophagy



Constraint

All germ-free mice are Balb/c

(or Swiss webster in S.16 and S.23)

All SPF mice are C57BL/6

(exception Figure 4: $RAG2^{-/-}$ Balb/c)

The concentration of SCFA depends on the colonisation status

Table S1. SCFA levels

Cecal or SI contents	SPF	ASF	GF	GF + P	GF + A	GF + B	GF + Mix	SPF +P	SPF + A	SPF + B	SPF + Vanco	SPF SI	SPF SI + P
mmol/g luminal contents													
Propionate	21.90 ± 0.122	18.83 ± 2.72	1.46 ± 0.162	14.93 ± 4.58	–	–	11.78 ± 6.38	43.33 ± 12.17	–	–	1.851 ± 1.41	2.69 ± 0.24	12.08 ± 3.69
Acetate	40.66 ± 5.86	28.69 ± 8.32	2.82 ± 0.534	–	16.18 ± 4.65	–	20.83 ± 0.47	–	53.95 ± 1.91	–	8.63 ± 1.73	15.19 ± 9.2	15.46 ± 3.69
Butyrate	18.52 ± 4.92	16.89 ± 1.46	2.13 ± 0.598	–	–	11.46 ± 1.46	20.6 ± 3.96	–	–	29.78 ± 6.202	1.04 ± 0.903	7.183 ± 2.39	6.45 ± 2.11

Select bacterial species	ASF 356 (XIV)	ASF 492 (XIV)	*Clostridium ramosum* (XVII)	*Clostridium bifermentans* (XI)	Bacteroides fragilis
mmol/10^5 CFU					
Propionate	62.39 ± 0.22	22.93 ± 0.109	14.74 ± 0.526	1.147 ± 0.008	0.0517 ± 0.001
Acetate	220.0 ± 0.435	123.2 ± 0.272	118.4 ± 0.526	1.973 ± 0.001	0.137 ± 0.001
Butyrate	ND	ND	ND	ND	ND

SCFA mix in mouse water bottle over time	Input (Day 0)	Day 1	Day 4	Day 7	Day 10	Day 14	Day 90
mM							
Propionate	200	200	199.6	201.24	202.9	203.2	41.9
Acetate	200	195	220.1	192.2	196.3	202.5	56.1
Butyrate	200	203.09	202.85	202.27	202.69	202.19	38

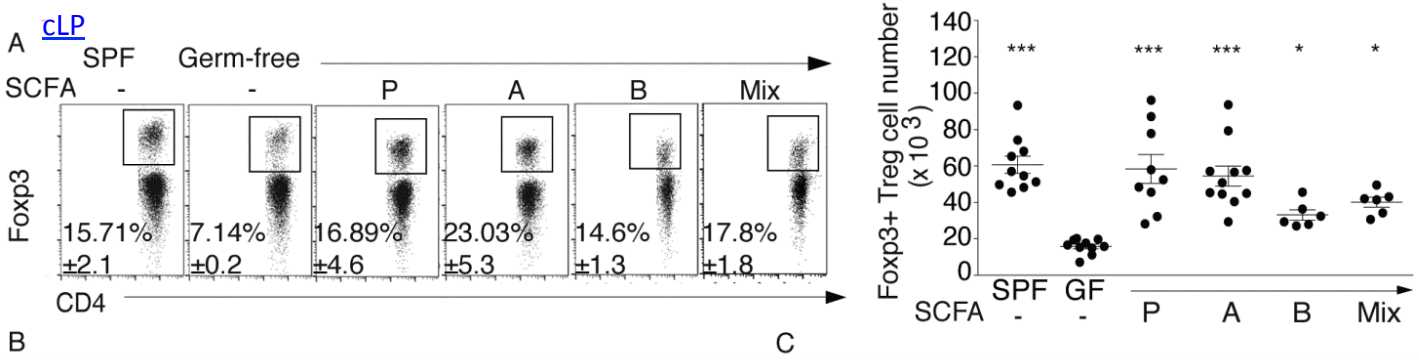
Cecal or SI contents	SPF	ASF	GF
mmol/g luminal contents			
Propionate	21.90 ± 0.122	18.83 ± 2.72	1.46 ± 0.162
Acetate	40.66 ± 5.86	28.69 ± 8.32	2.82 ± 0.534
Butyrate	18.52 ± 4.92	16.89 ± 1.46	2.13 ± 0.598

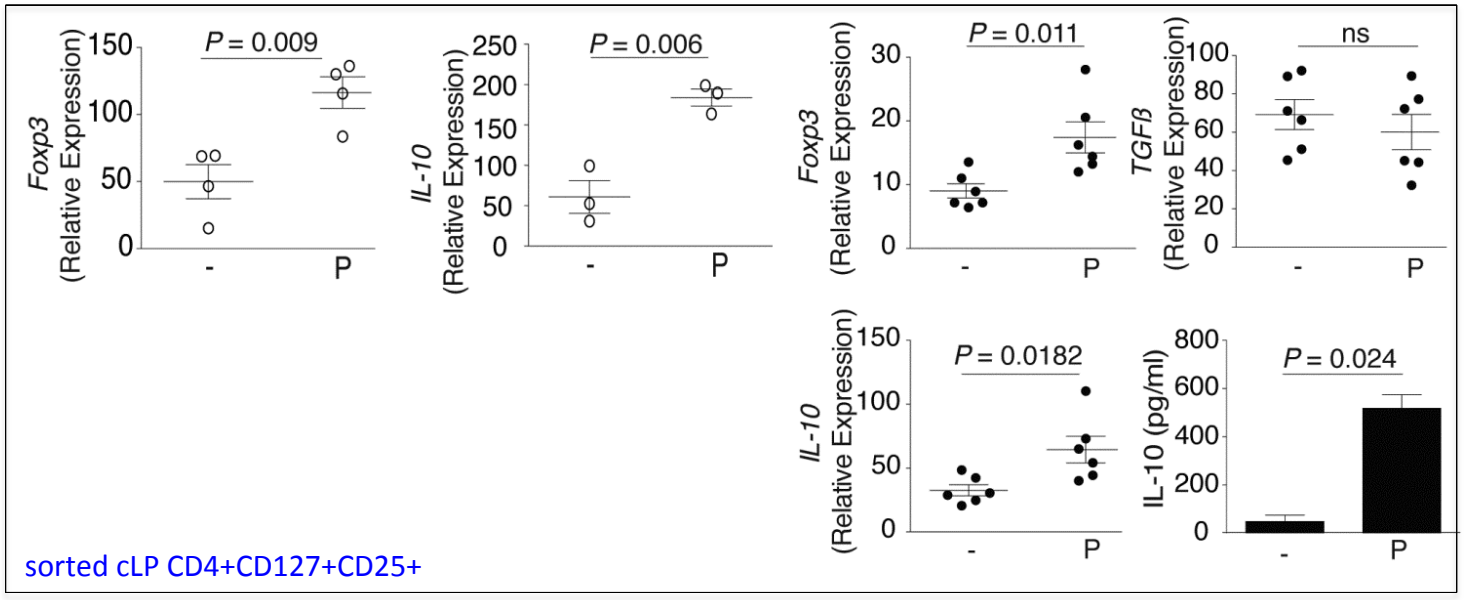
GF

SCFA restore colonic Treg populations and fuction in germ-free mice

Mice were treated for 21 days with either
sodium acetate (150mM),
sodium propionate (150mM),
sodium butyrate (100mM) or a
SCFA mix (67.5mM acetate, 40mM Butyrate, 25.9mM Propionate)
in the drinking water and water solutions were prepared and changed weekly.
Controls: mice received pH and sodium-matched water

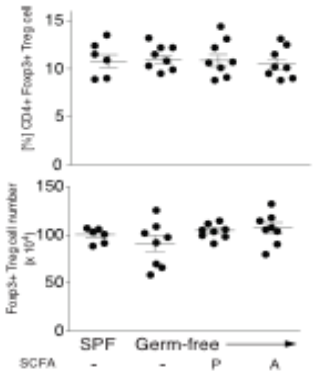
cLP



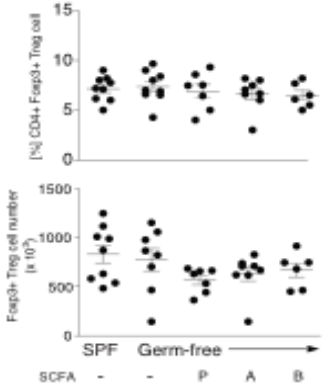


sorted cLP CD4+CD127+CD25+

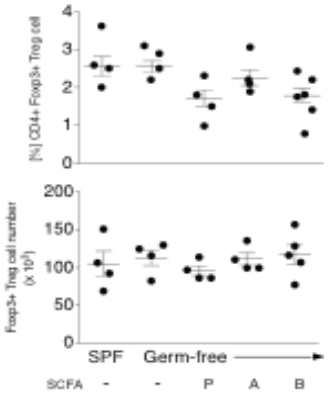
Spleen



MLN

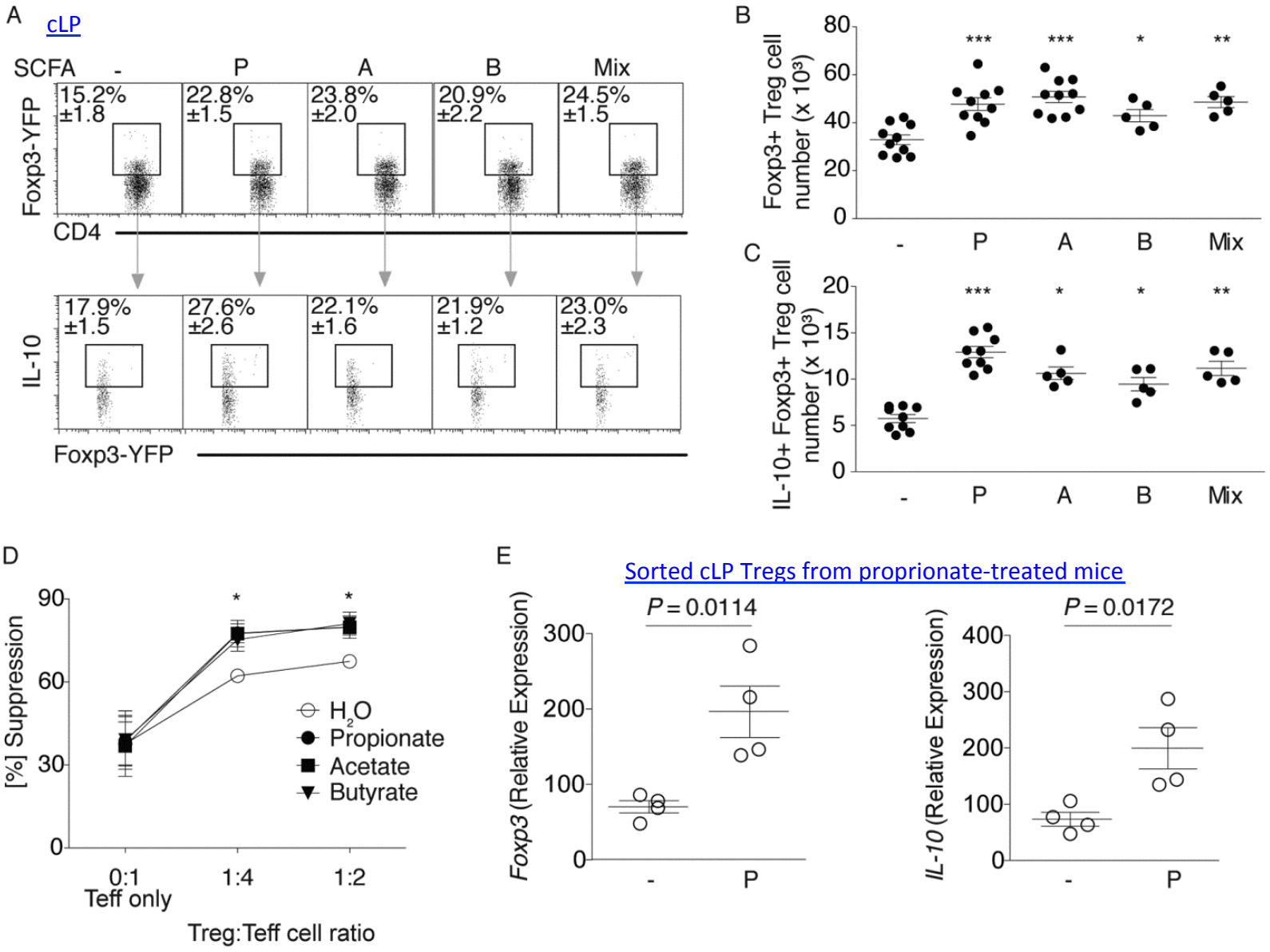


Thymus

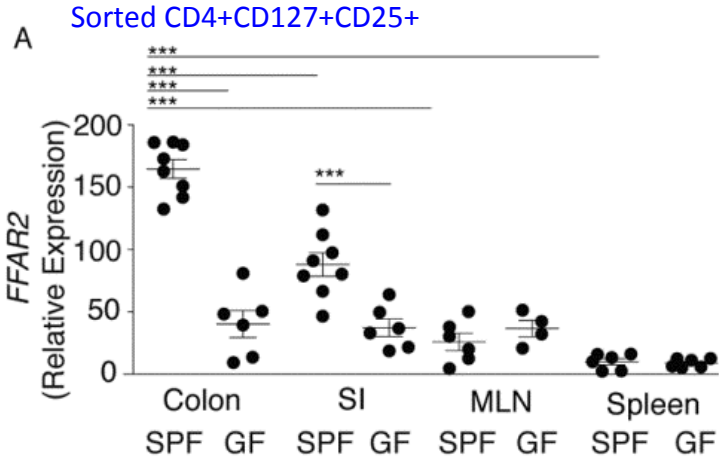


SPF

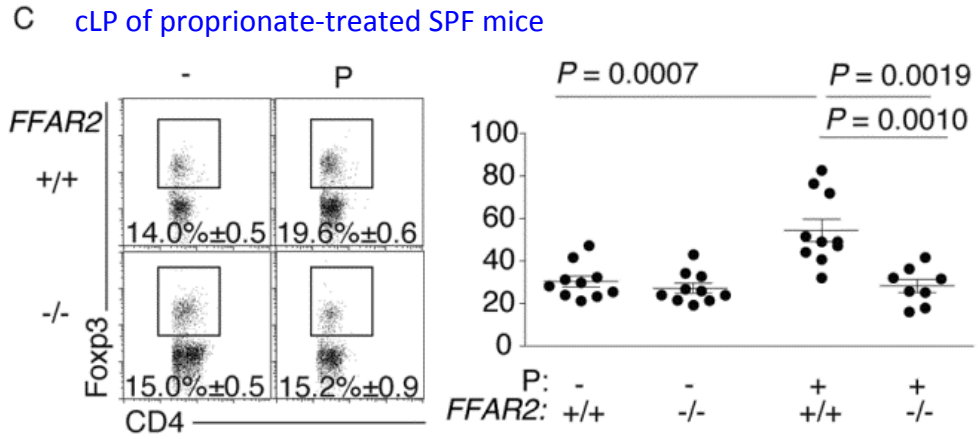
SCFA augment colonic Treg population size and function in SPF mice

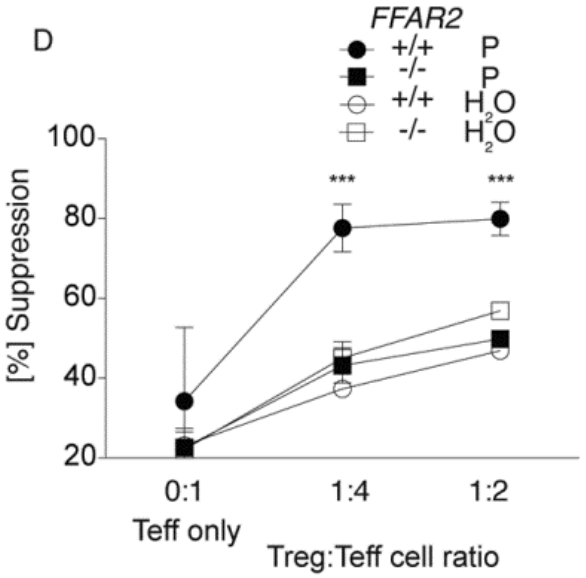


GPR43 mediates SCFA effects on cTregs

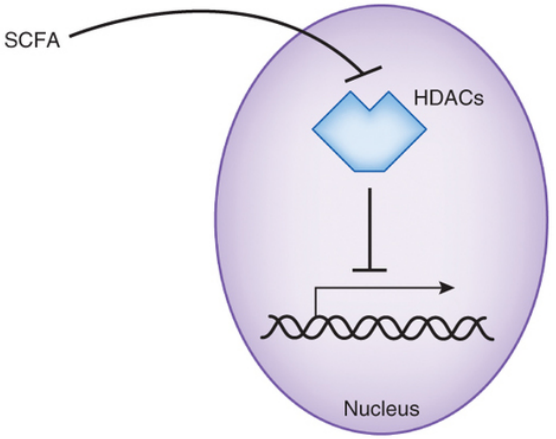


Microbiota-dependent expression of FFAR2

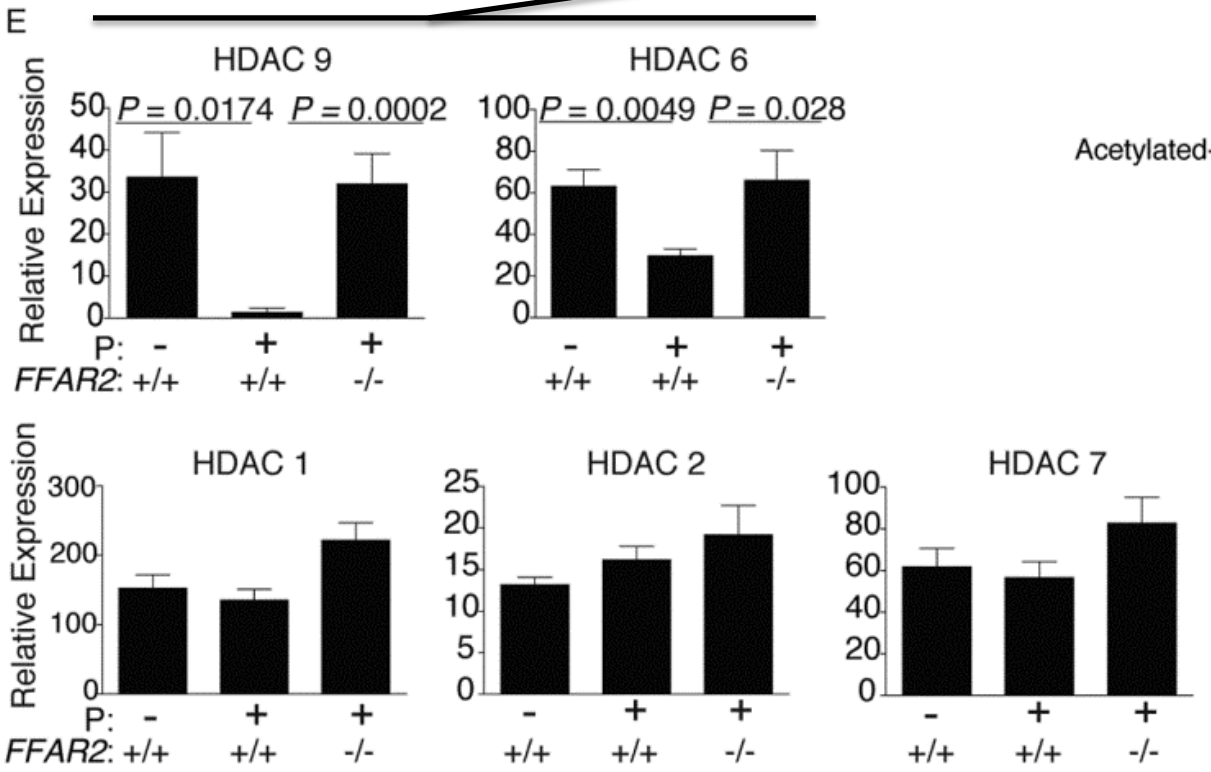


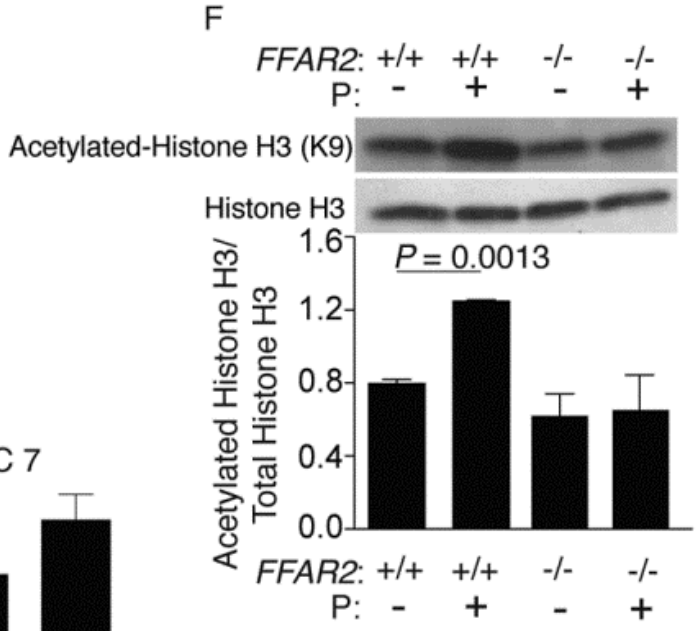


Inhibition of Histone-deacetylases contributes to SCFA effect on Tregs



HDAC6/9 can downregulate nTregs
(Beier U.H., 2012)





SCFA exposure ameliorates T cell transfer colitis

(Treg-intrinsic, GPR43-dependent)

A-C: Balb/c SPF

D-F: C57BL/6 SPF