The aryl hydrocarbon receptor: dichotomously linking environmental signals to immune suppression and autoimmunity

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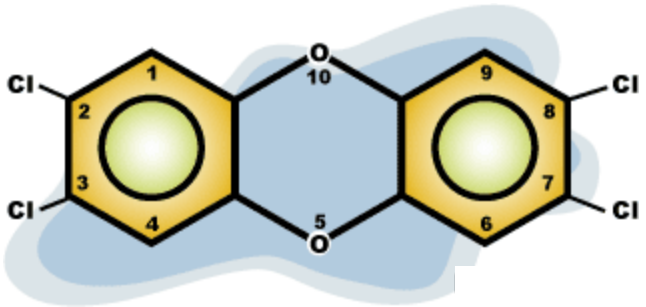
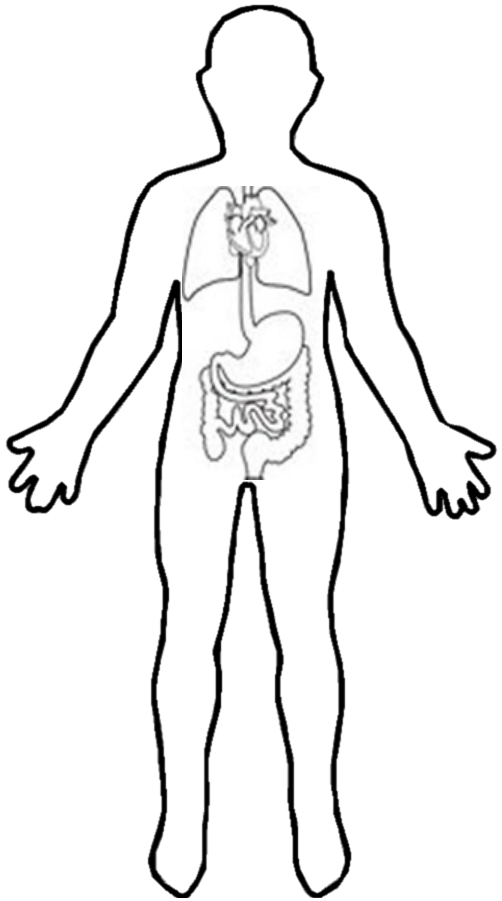


UCDAVIS
UNIVERSITY OF CALIFORNIA

Aryl hydrocarbon receptor (AhR): An environmental sensor

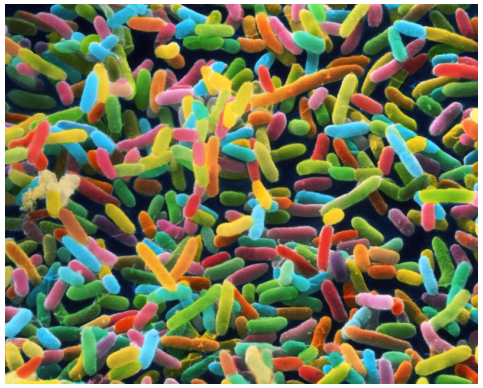
Environmental



Dietary

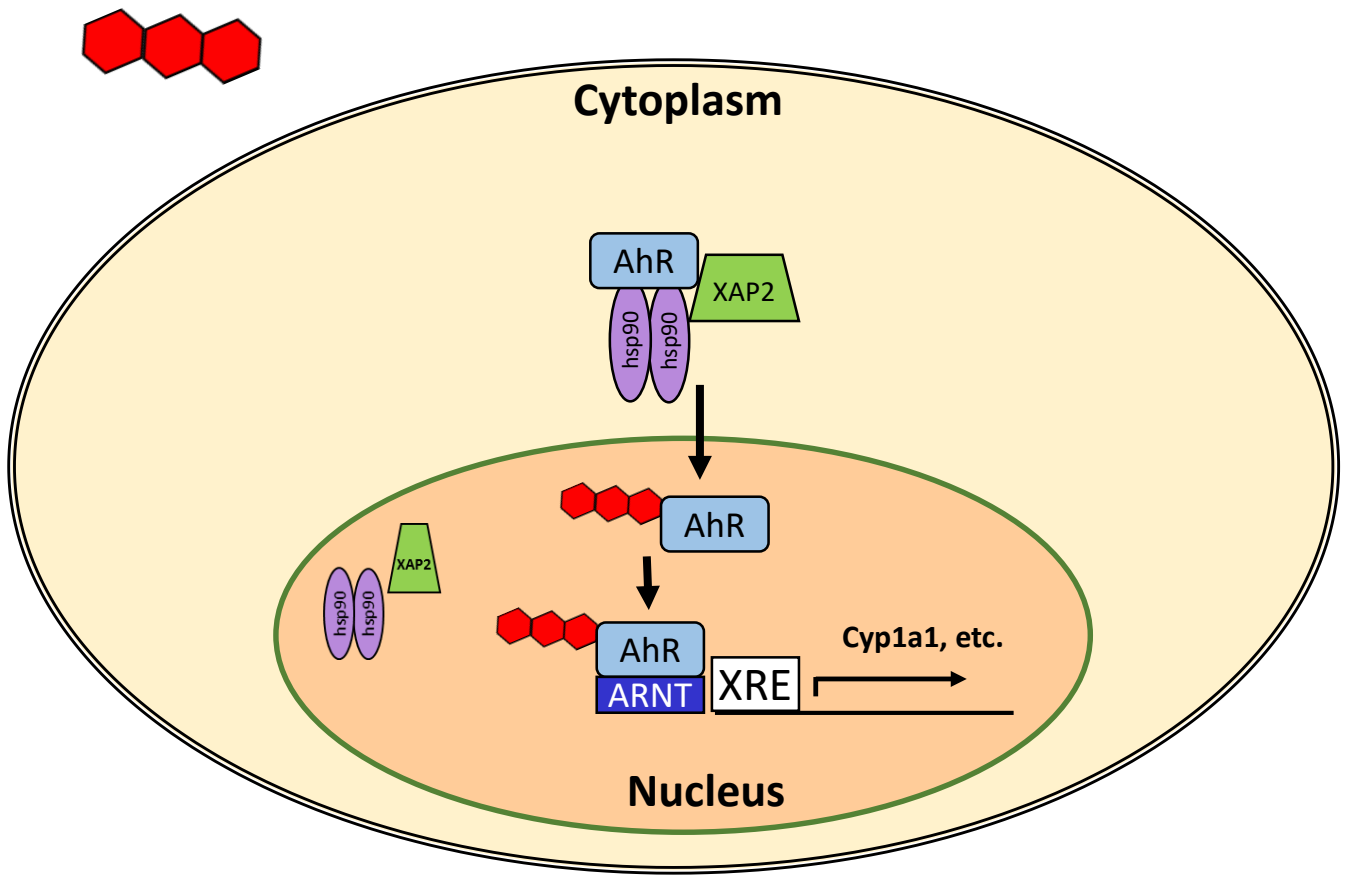
Pharmacologic

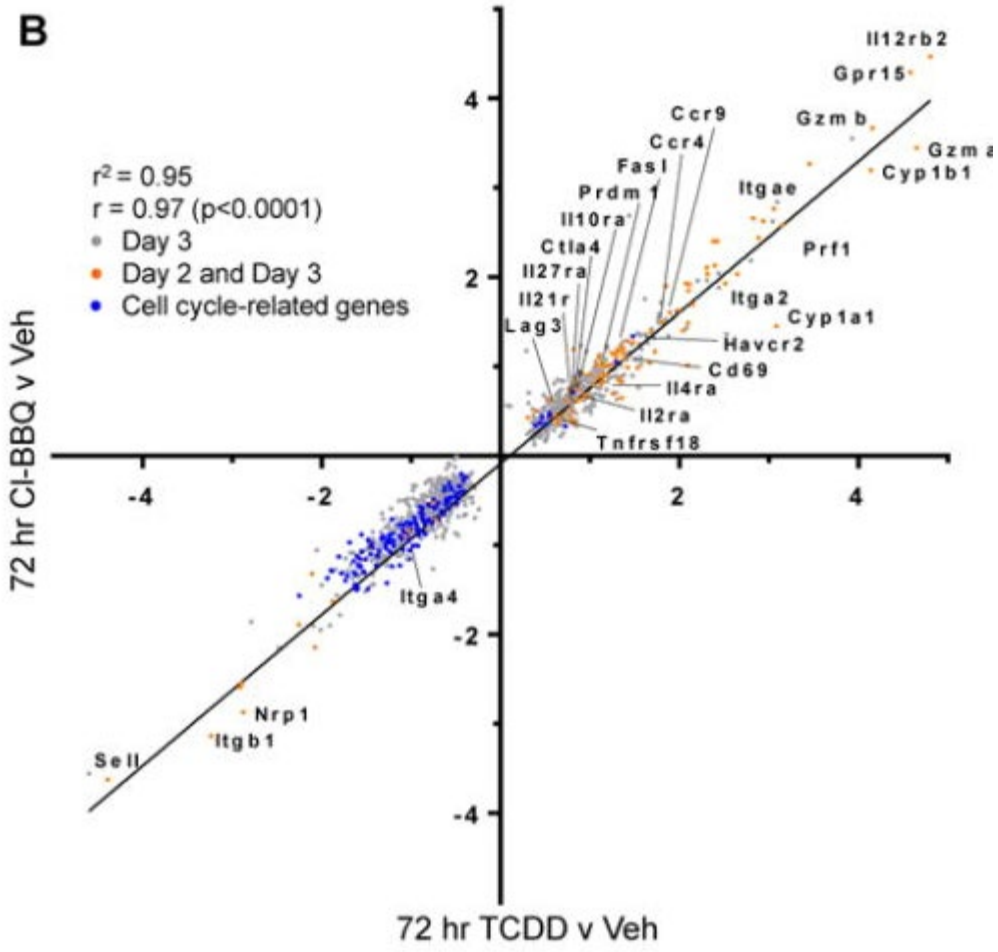
Bacterial Derived



AhR is a ligand activated transcription factor

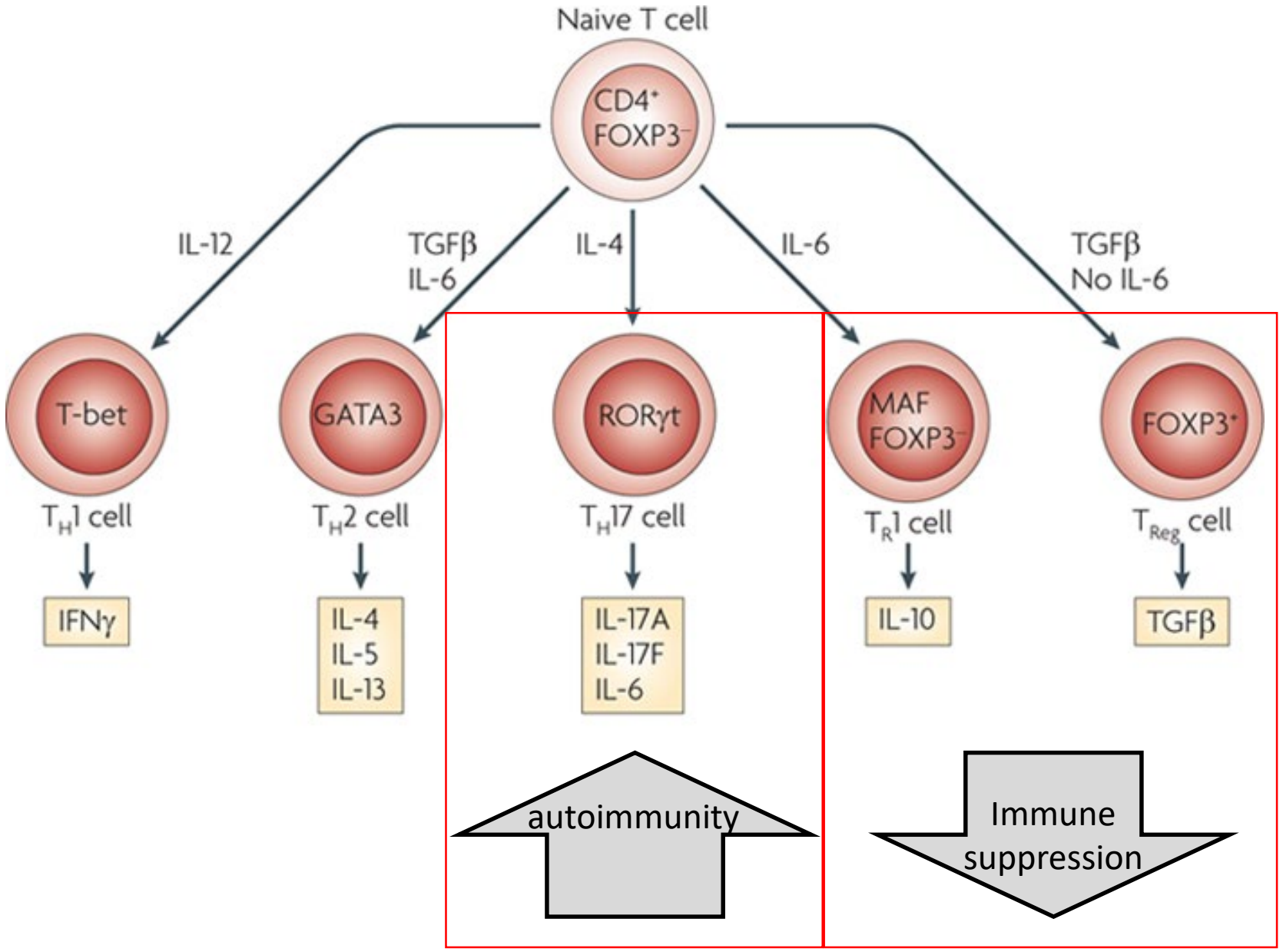
- A variety of immune cells, **including CD4⁺ T cells**, express AhR.





Ehrlich A et al (2017) Eur J Immunol. 47(11):1989-2001.

CD4+ T cells orchestrate immune response outcomes

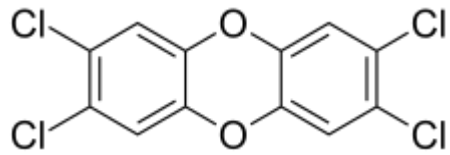


Nat Rev Immunol. 2010 Mar;10(3):159-69

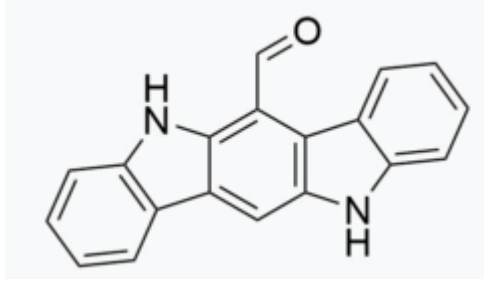
AhR ligands can drive opposing T cell fates

- How do AhR impact CD4+ T cell responses during EAE (mouse model of MS)?

- TCDD has well established immunosuppressive activity
  - Long half life



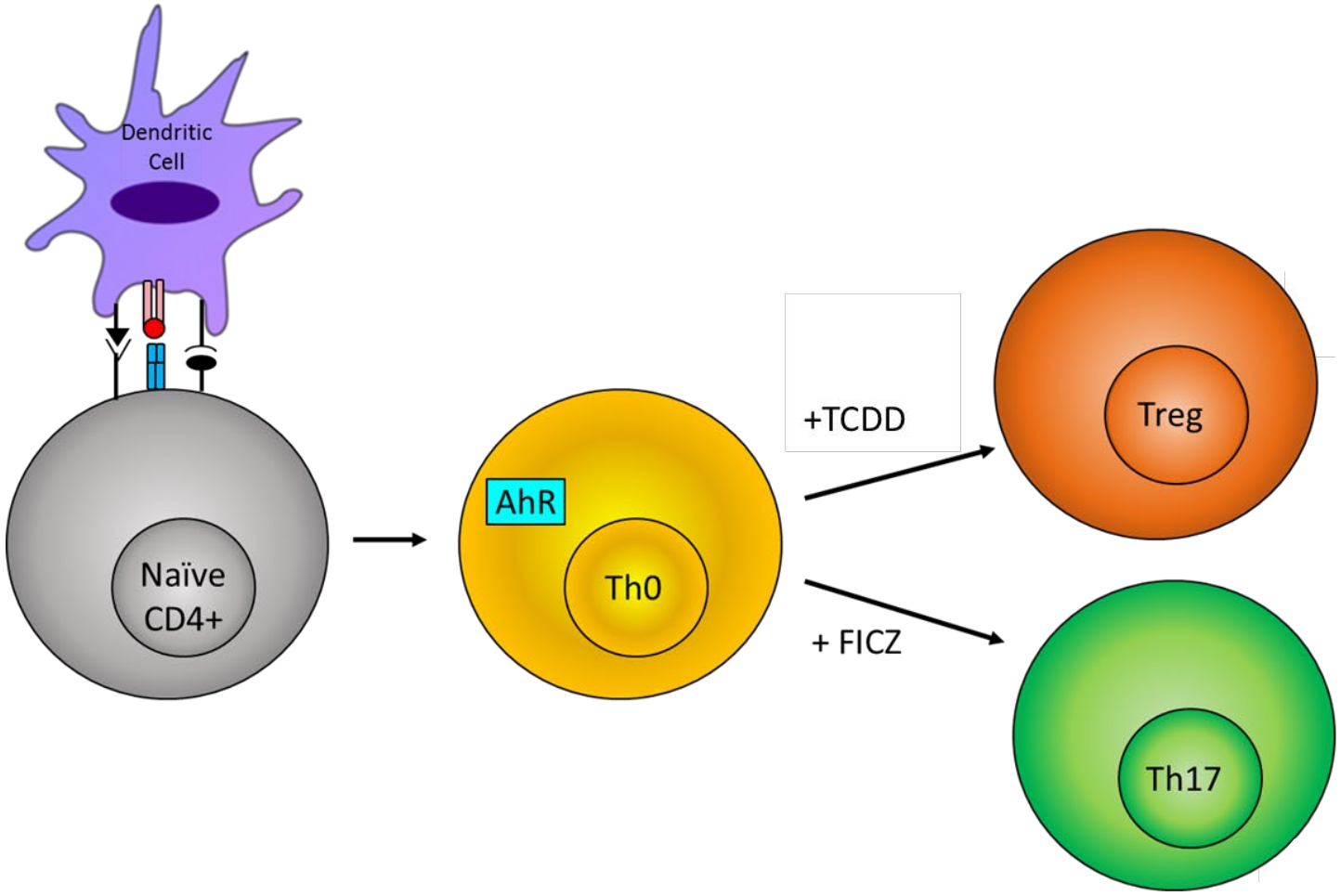
- FICZ can be naturally found in cell culture culture media and skin (Trp + UVB)
  - Rapidly metabolized

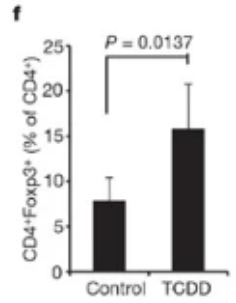


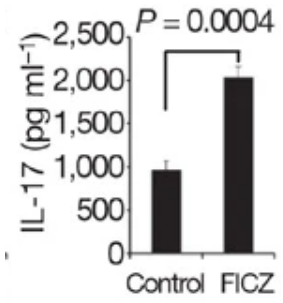
- Both TCDD and FICZ are AhR agonists

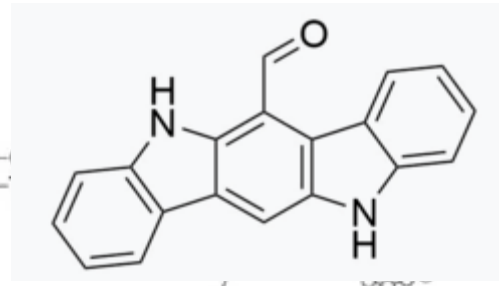
Quintana, F., Basso, A., Iglesias, A. et al. Control of Treg and TH17 cell differentiation by the aryl hydrocarbon receptor. Nature 453, 65–71 (2008). https://doi.org/10.1038/nature06880

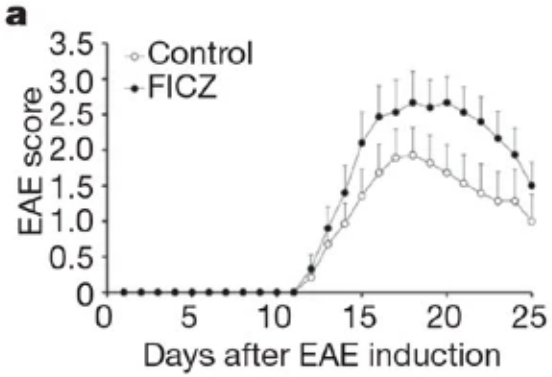
AhR ligands can drive opposing T cell fates











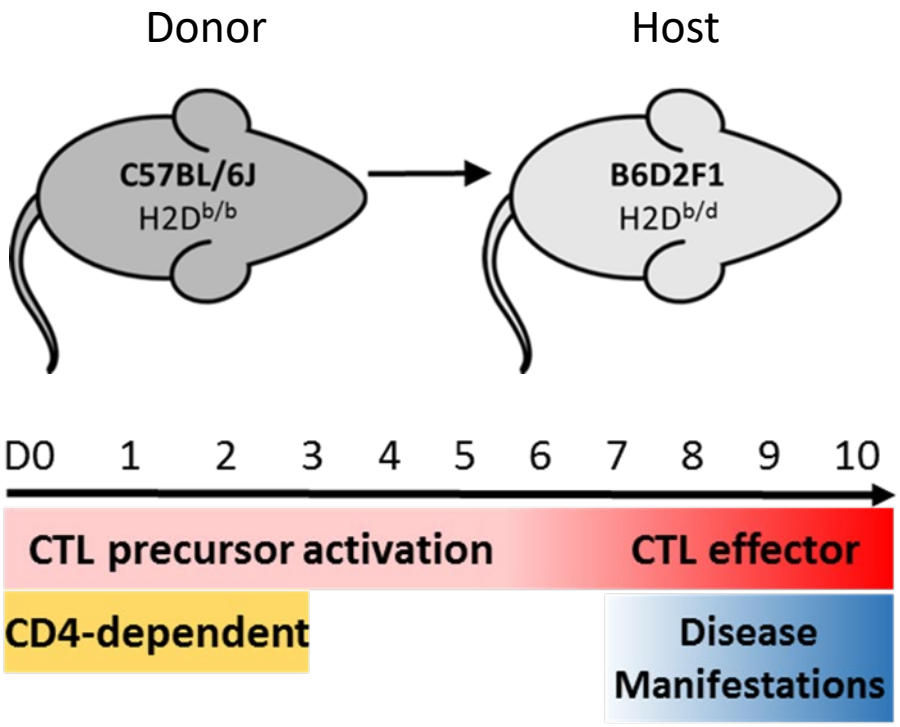
Quintana, F., Basso, A., Iglesias, A. et al. Control of Treg and TH17 cell differentiation by the aryl hydrocarbon receptor. Nature 453, 65–71 (2008). https://doi.org/10.1038/nature06880

How does activation of the same receptor lead to opposing outcomes?

Story #1: AhR-mediated modulation of CD4+ T cell differentiation is dependent on ligand dose, half-life, and receptor affinity (alloresponse model)

Story #2: AhR-mediated modulation of CD4+ T cell differentiation is dependent on tissue site (type 1 diabetes model)

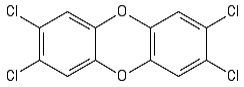
Parent-into-F1 alloresponse model

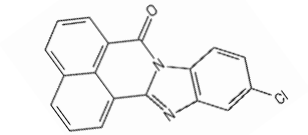


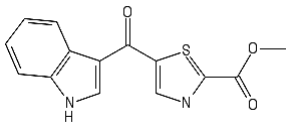
Donor T cells recognize Host antigen (presented by DCs) as foreign
CD4+ T cells prime CD8+ cells to become cytotoxic (CTL)
Donor CTLs destroy host cells (B cells as a readout)
Host tissue destruction and weight loss

Reported AhR ligand dosing regimens

Treg Induction

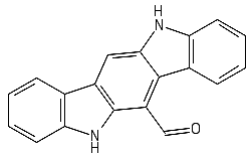






Ligand	Dose	Regimen	Route	Selected References
TCDD	15µg/kg 50µg/kg 15µg/kg 50µg/kg 50µg/kg*	1x Biweekly 1x 1x 1x	gavage gavage gavage i.p. i.p.	Funatake, J. Immunol., 2005; Kerkvliet, Immunotherapy, 2009; Schulz, Tox. Sci., 2012; Pauly, Toxicol. Environ. Chem., 2012; Quintana, Nature, 2008
Cl-BBQ	10mg/kg 60mg/kg	1x/day 3x/week	i.p. gavage	Punj, PLoS One, 2014; Ehrlich, J. Immunol., 2016
ITE	10mg/kg* 10mg/kg*	1x/day 1x/day	i.p. i.p.	Quintana, PNAS, 2010; Nugen, IOVS, 2013

Th17 Induction



Ligand	Dose	Regimen	Route	Selected References
FICZ	50µg/kg* 30µg/kg 50µg/kg 100µg/kg 50µg/kg	1x 1x 1x/3-4d d-1 and 4 1x	i.p. s.c. i.p. i.p. gavage	Quintana, Nature, 2008; Veldhoen, Nature, 2008; Schulz, Tox. Sci., 2012; Pauly, Toxicol. Environ. Chem., 2012; Signh, J. Immunol, 2016

DOCKING SCORE

	H AHR	M AHR
TCDD	-24.2	-22.15
ITE	-11.44	-13.43
FICZ	-11.38	-11.24
11BBQ	-10.98	-13.09

Ehrlich, et al. Toxicol Sci. 2018

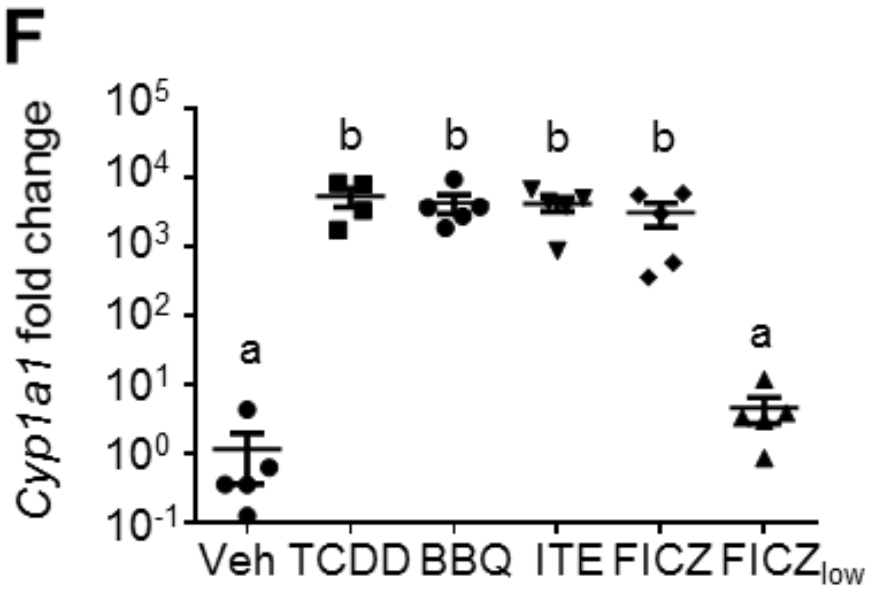
Hypothesis

If administered at concentrations that equivalently induce and maintain AhR activation, different AhR ligands will induce similar effects on T cell activation/differentiation.

Dose Determination

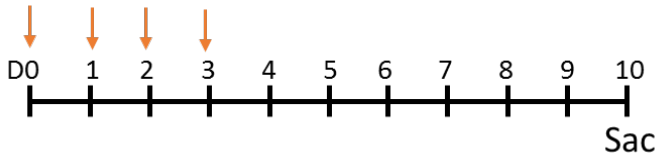
Doses were determined empirically, starting at 10mg/kg and adjusted based on *Cyp1a1* readout

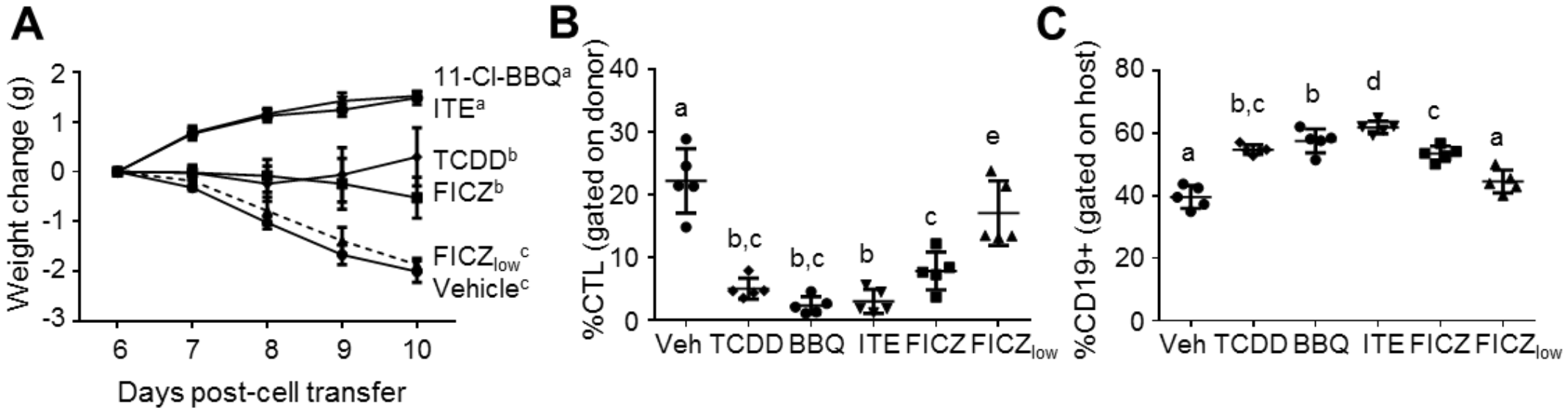
TCDD: 15µg/kg 1x
11-Cl-**BBQ**:7.5mg/kg 1x/day
ITE: 40mg/kg 4x/day
FICZ:10mg/kg 1x/day



Ehrlich, et al. Toxicol Sci. 2018

FICZ suppresses GVHD if administered at a dose that activates AhR equivalently to TCDD

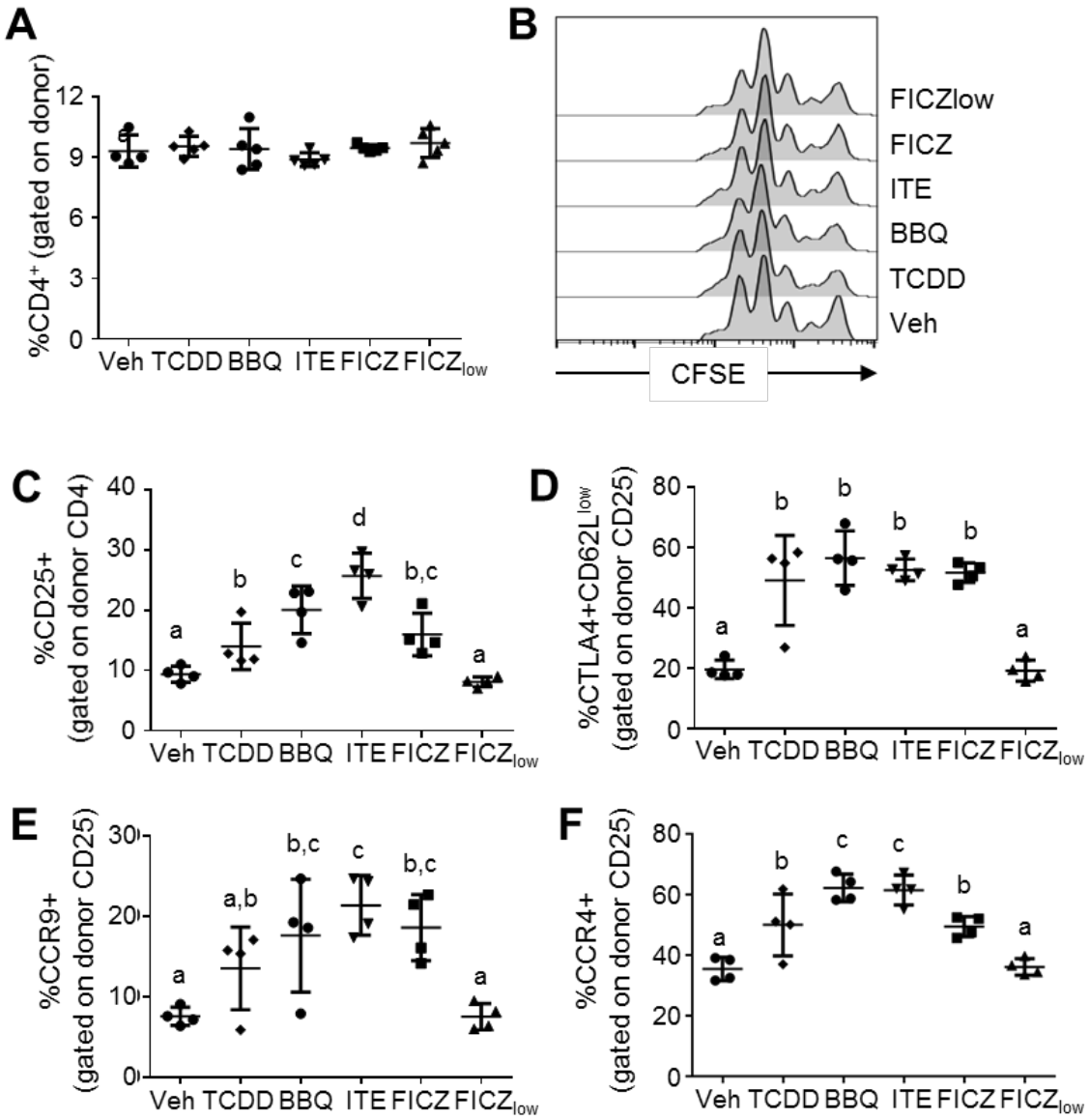




Ehrlich, et al. Toxicol Sci. 2018

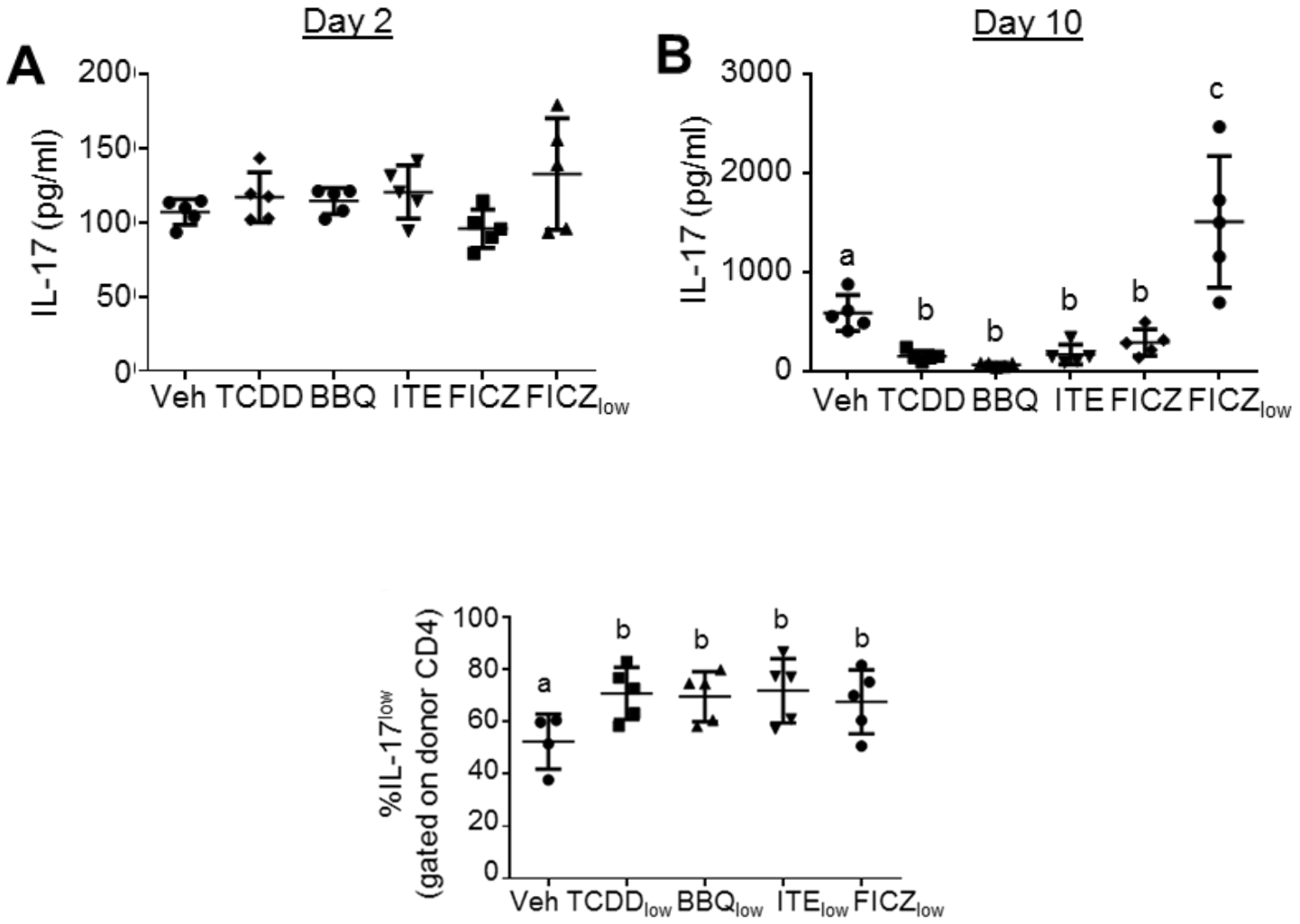
FICZ induces Tr1 cells when administered at doses that activate AhR equivalently to TCDD

Day 2



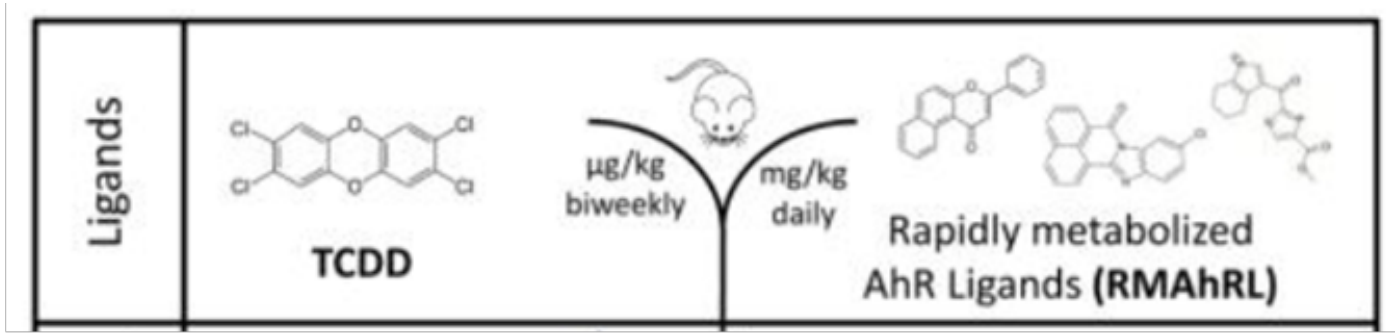
Ehrlich, et al. Toxicol Sci. 2018

Low dose FICZ administration induces IL-17

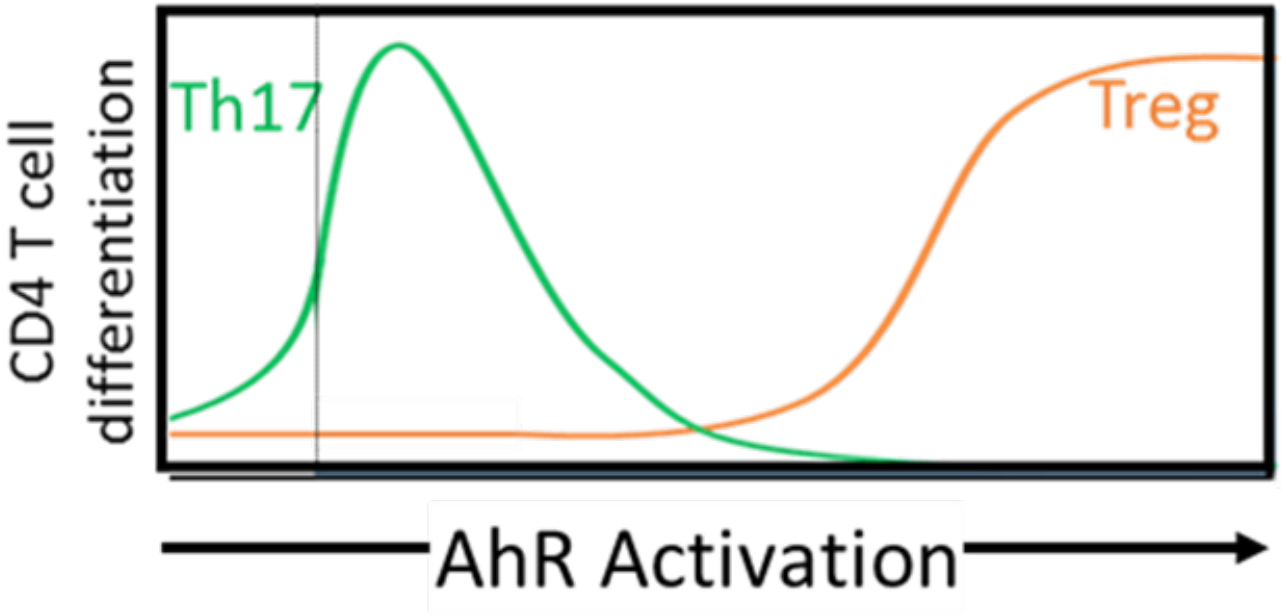


Ehrlich, et al. Toxicol Sci. 2017

CD4+ T cell fate is dependent on the extent of AhR activation



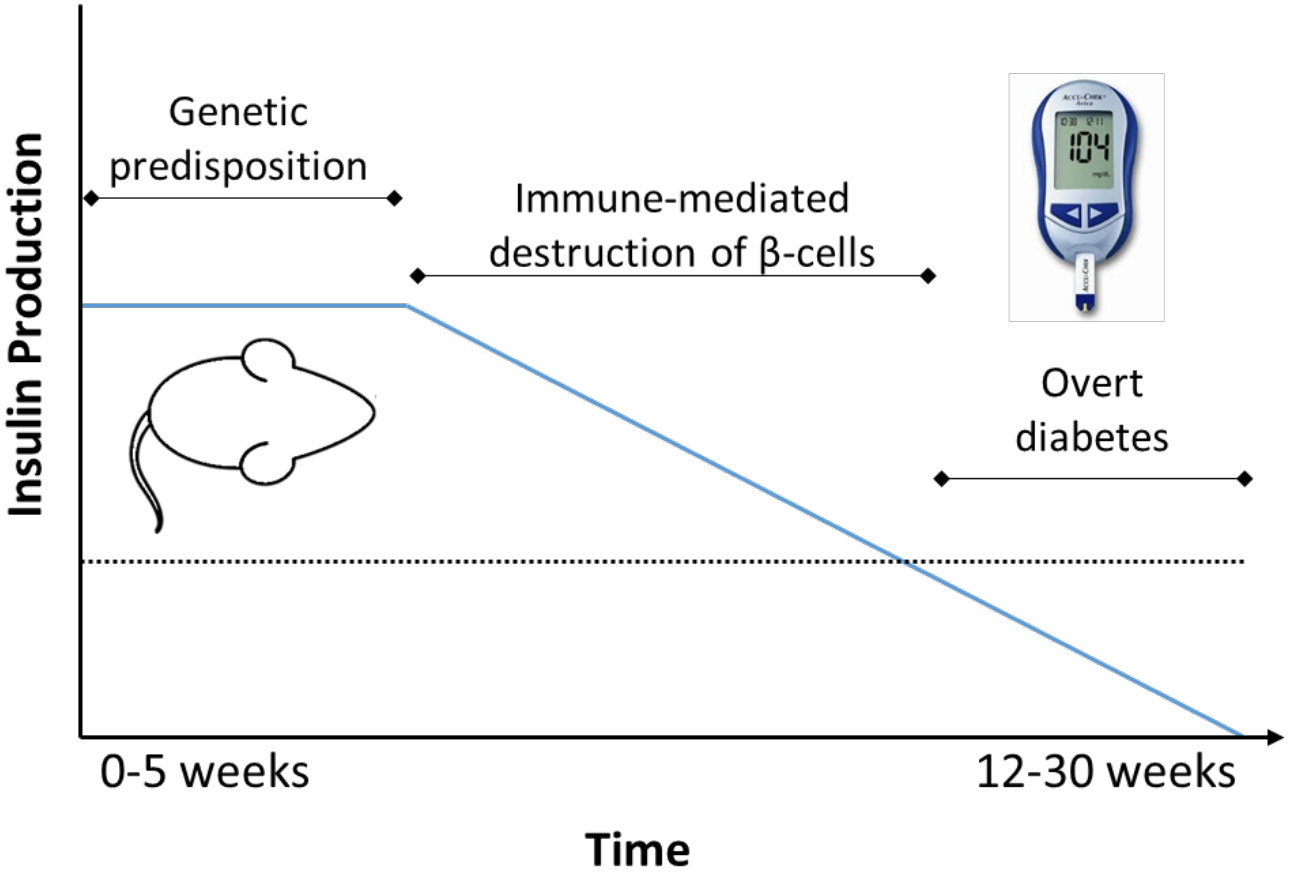
Ehrlich, A. and Kerkvliet, N. (2017) Current Opinion in Toxicology. 2:72-78.

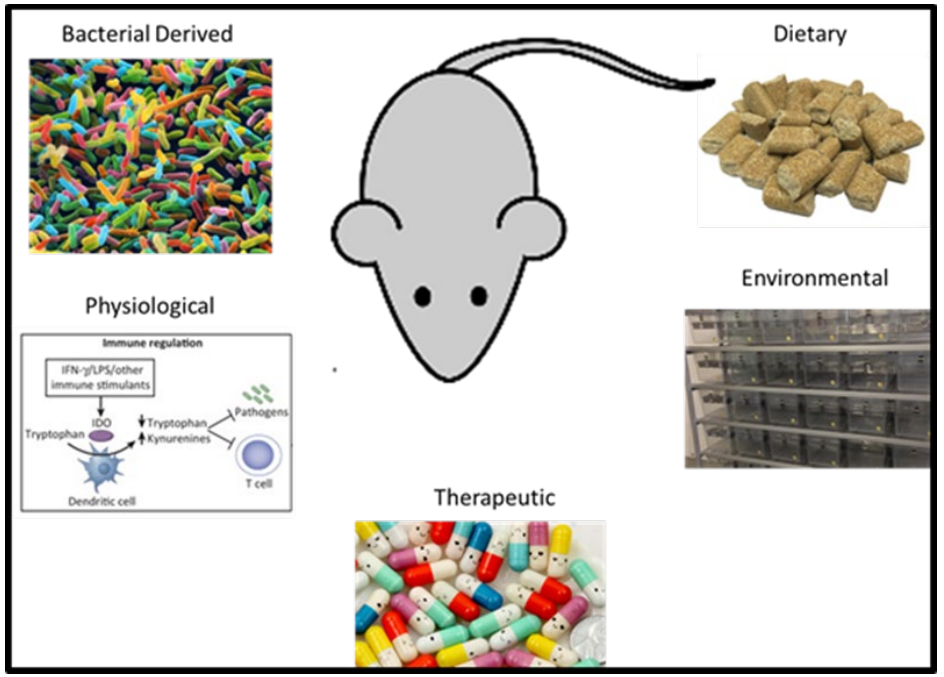


Ehrlich A. et al. (2018) Toxicol Sci. 161(2):310-320.

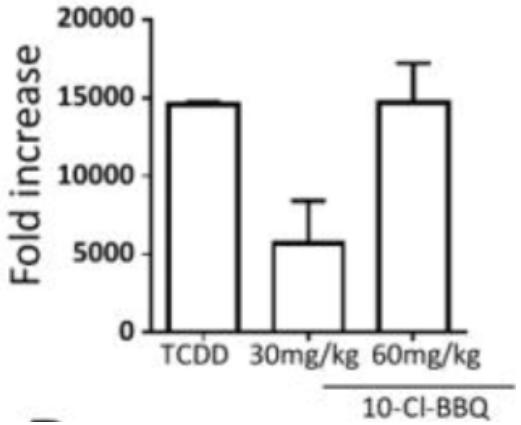
Hypothesis: Strong AhR activation will suppress the development of T1D

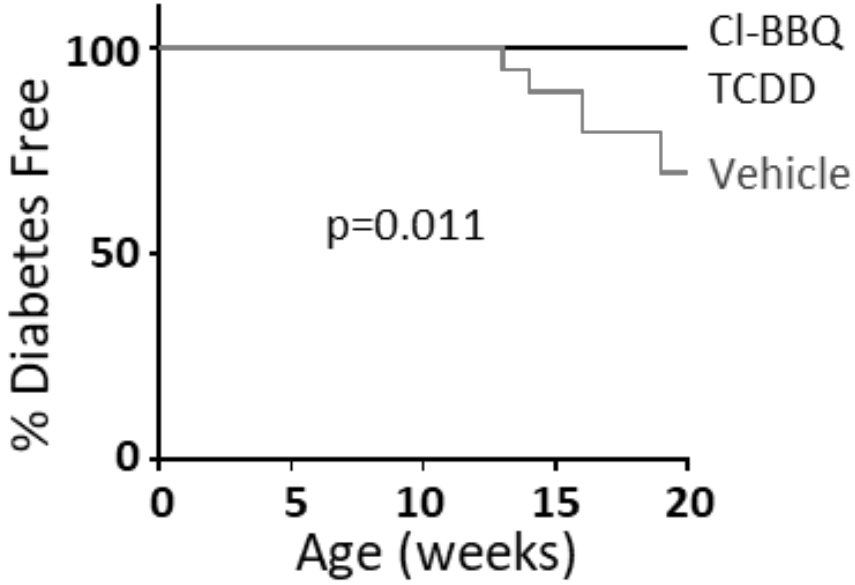
Diabetes incidence in NOD mice is dependent on housing environment

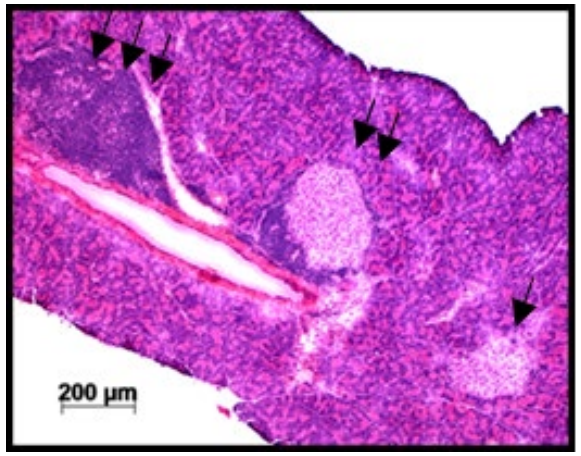




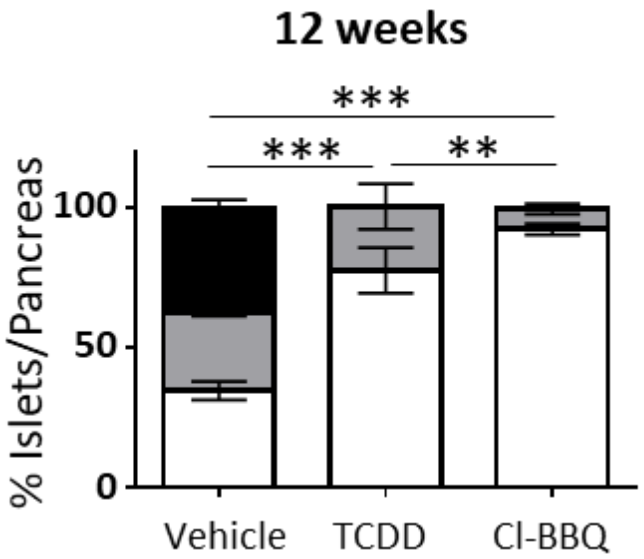
Strong AhR activation suppresses insulitis

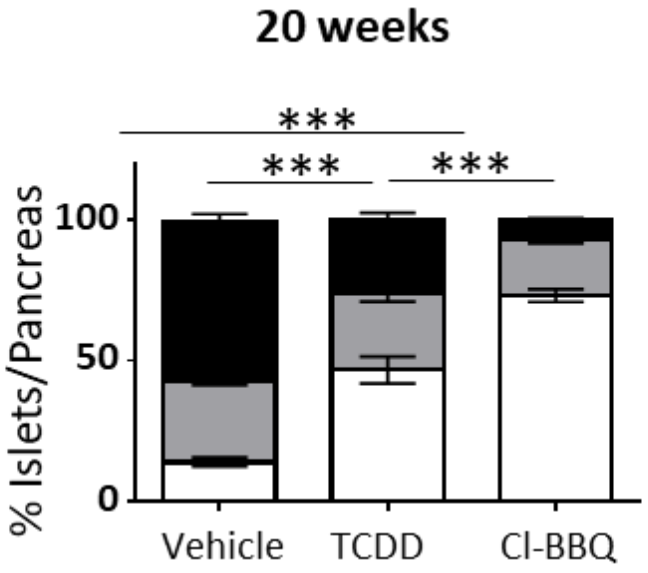


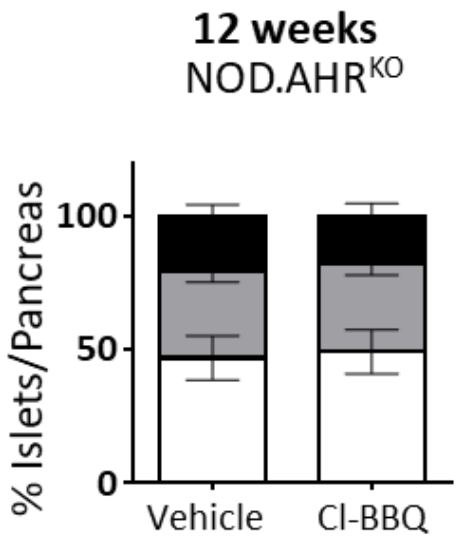




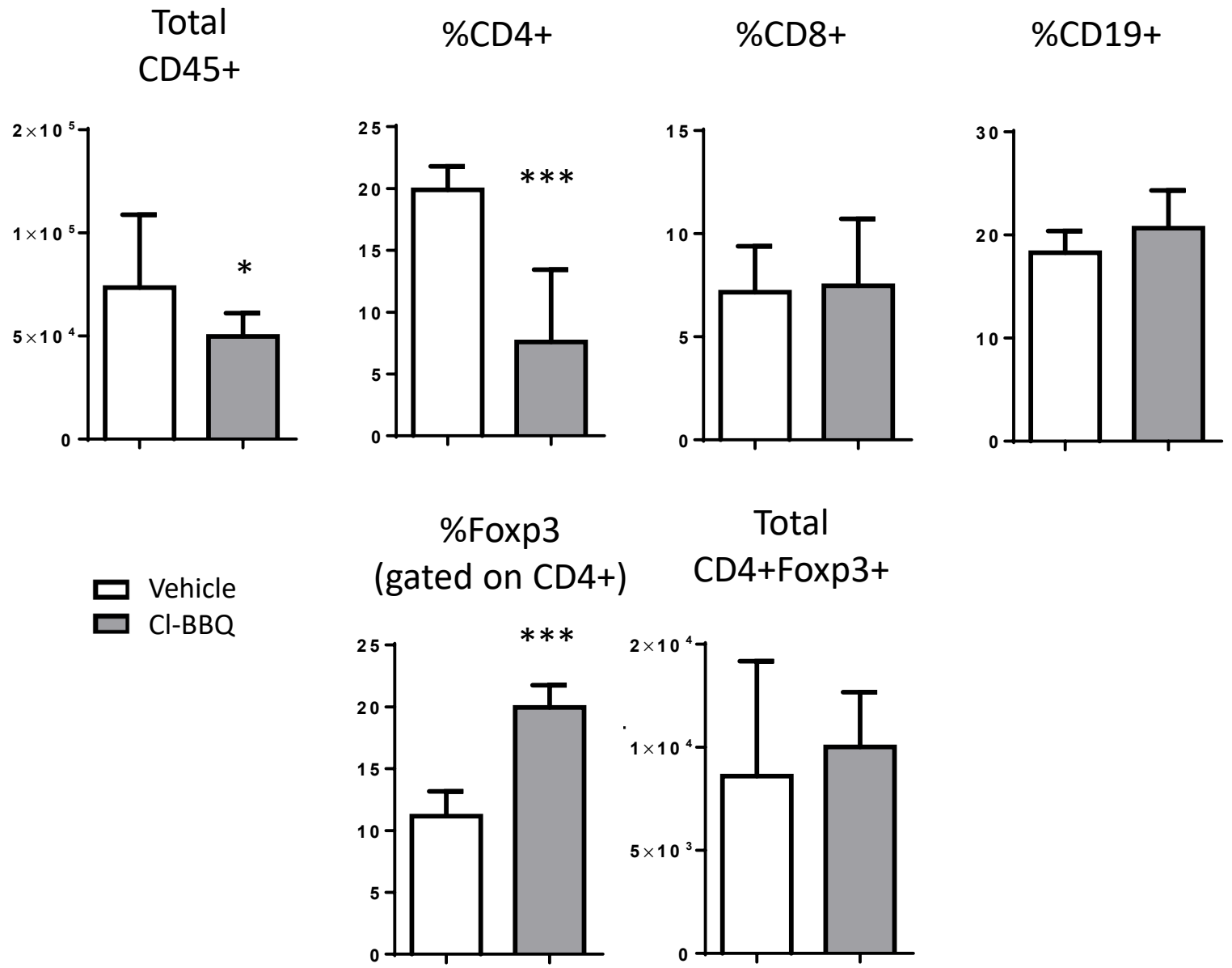
≥50% infiltration
<50% infiltration
No infiltration



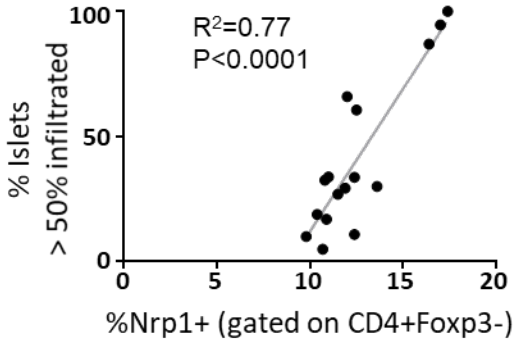


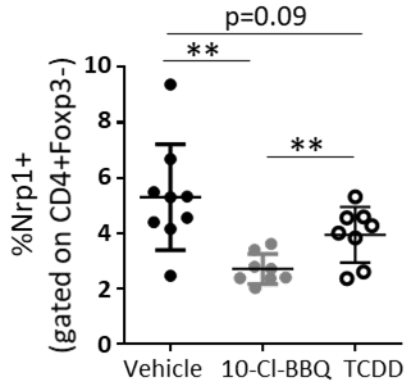


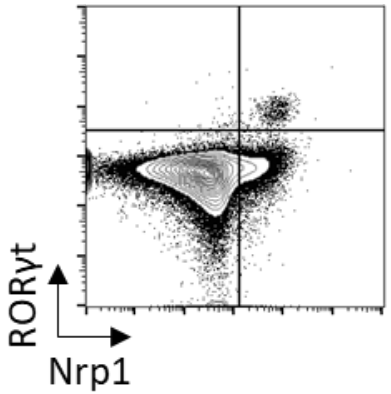
Strong AhR activation increases the % of pancreatic Tregs

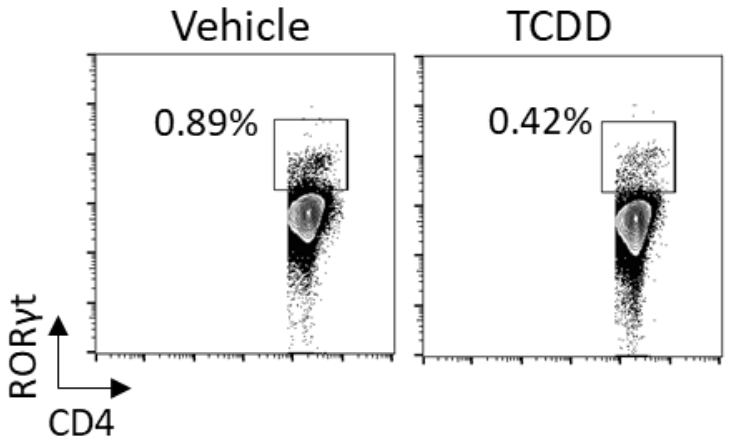


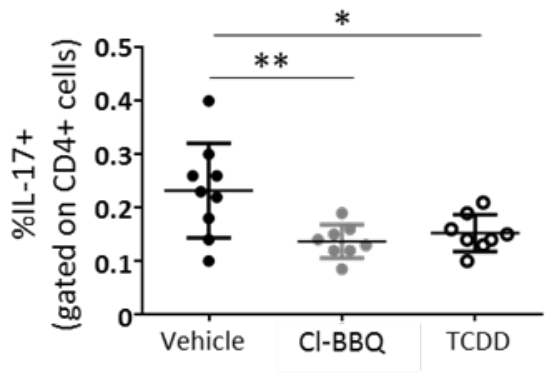
Strong AhR activation suppresses Th17 cells

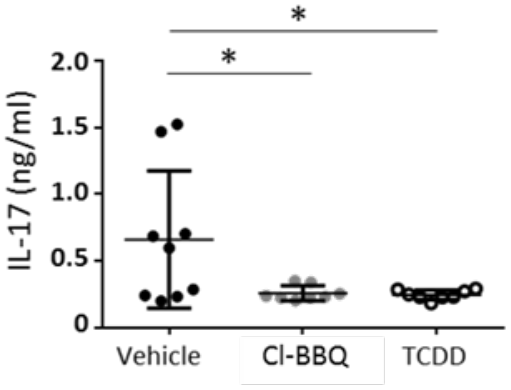






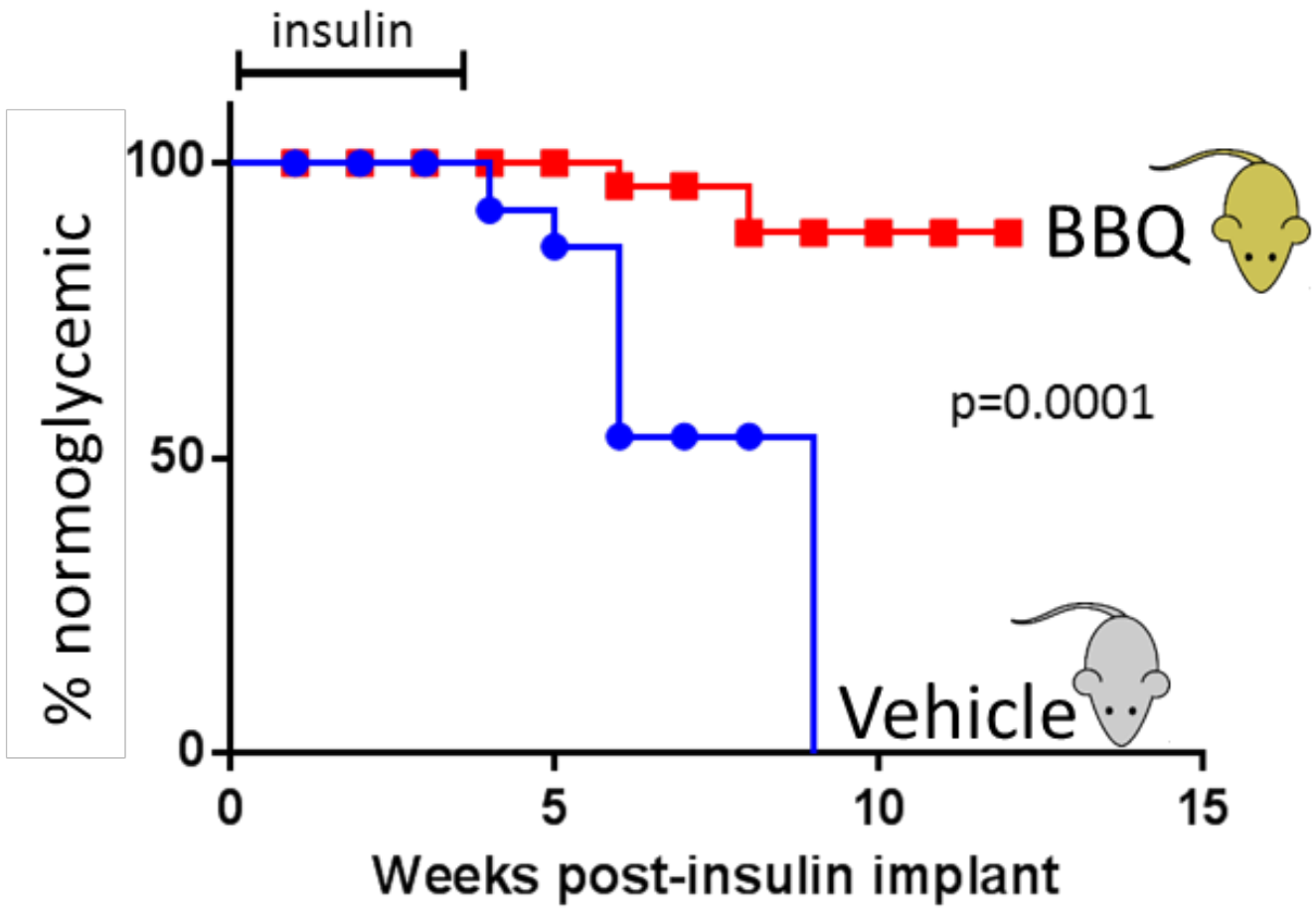






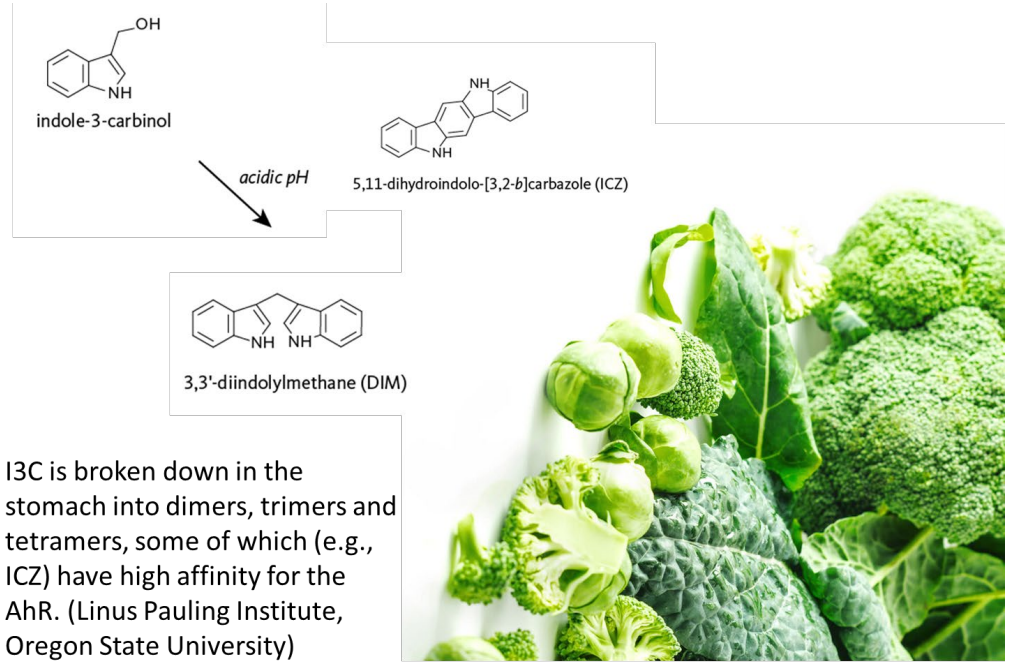
Ehrlich, et al. J Immunol. 2016 Jan 1; 196(1): 264–273.

Strong AhR activation “treats” T1D



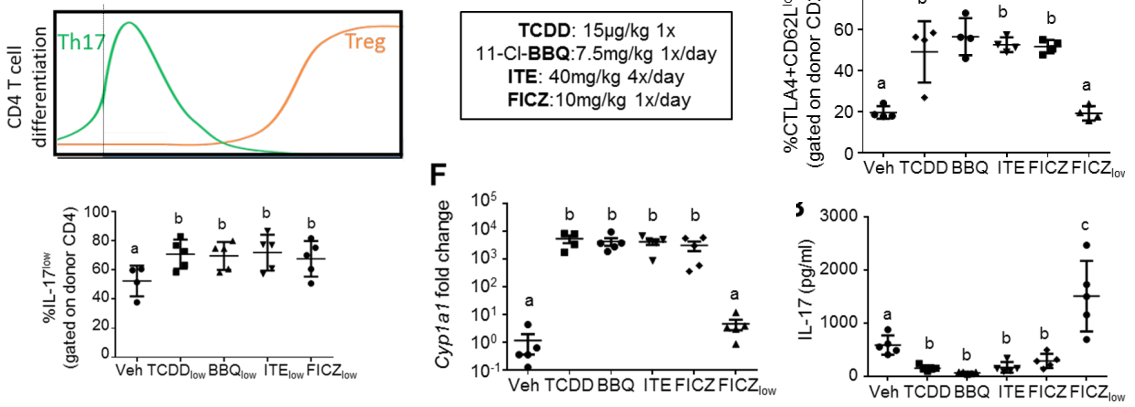
What about dietary AhR ligands?

Hypothesis: When given a **high dose** of the dietary AhR ligand precursor, indole-3-carbinol, NOD mice will have reduced insulitis, mimicking studies with CI-BBQ.

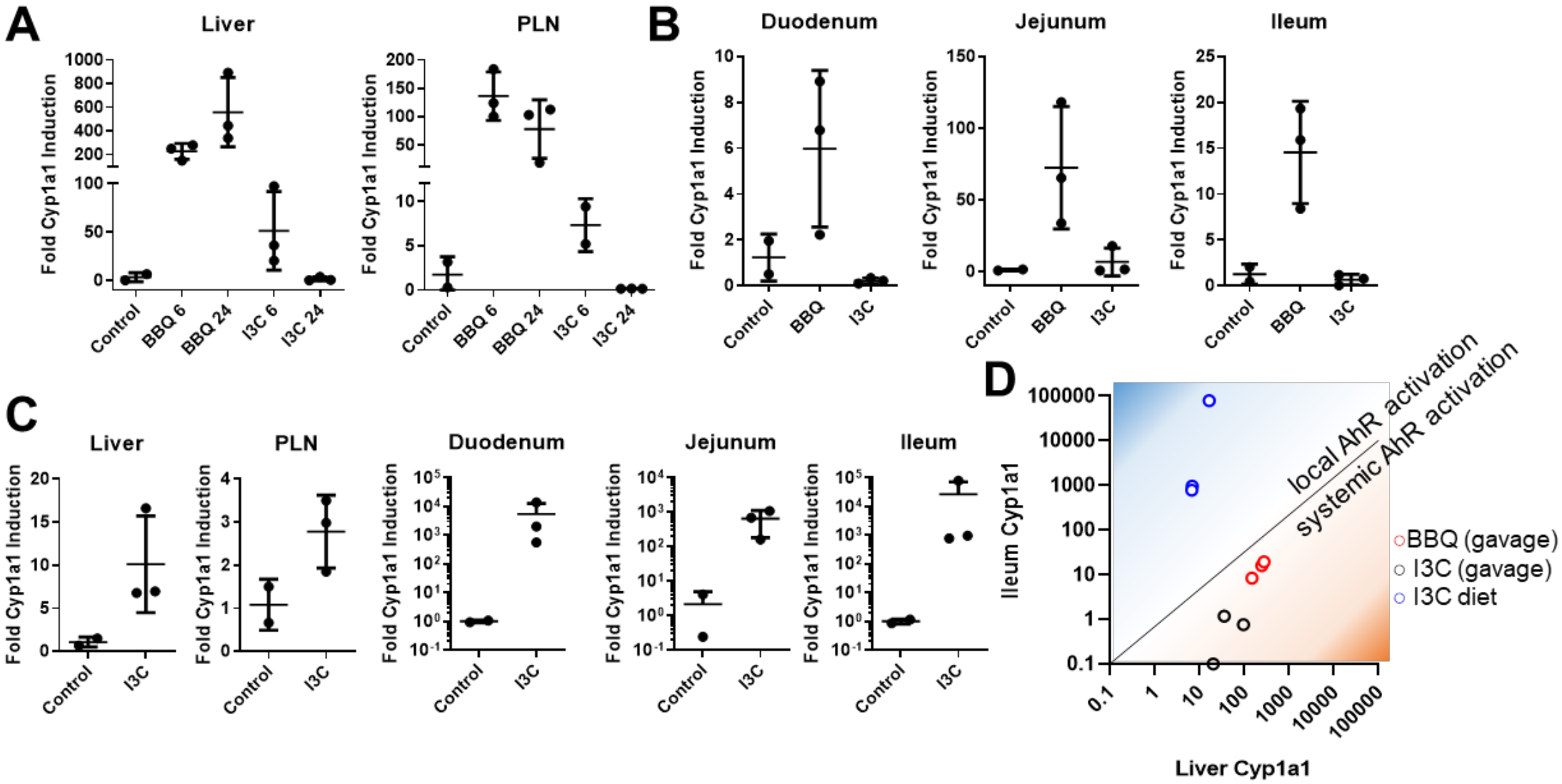


I3C is broken down in the stomach into dimers, trimers and tetramers, some of which (e.g., ICZ) have high affinity for the AhR. (Linus Pauling Institute, Oregon State University)

- In a GVH mouse model, different AhR ligands induce similar, dose-dependent, CD4+ T cell differentiation outcomes if AhR activation is normalized
- NOD mice a lower sensitivity AhR allele (AhR^d) (~10-fold, compared to C57BL/6 mice AhR^b)
- In other C57BL/6 mouse models of inflammatory diseases, dietary I3C (100-2000ppm), promotes Tregs, decreases effector CD4 T cells, reduces immunopathology

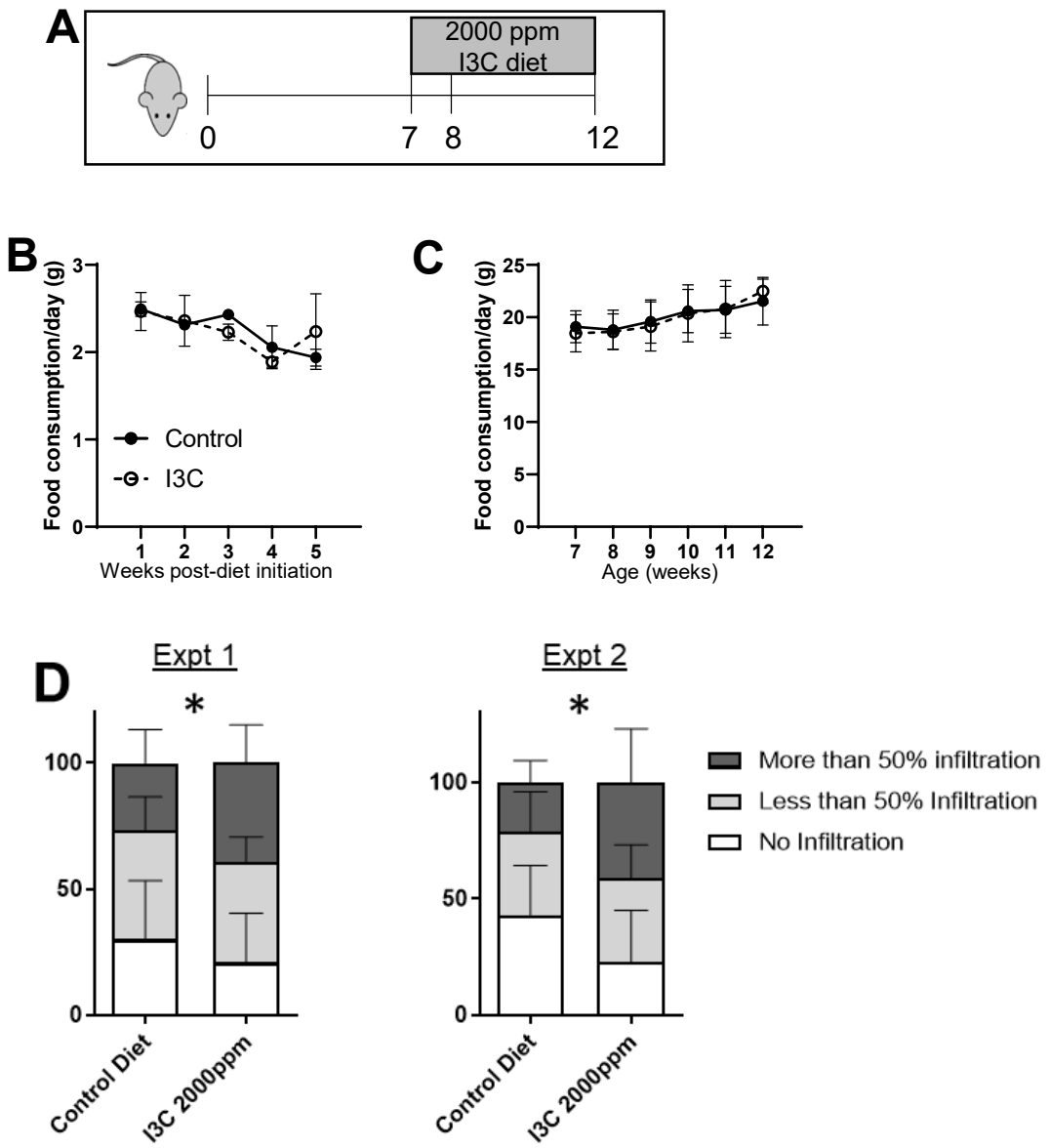


I3C strongly activates AhR in the intestine (not systemically)



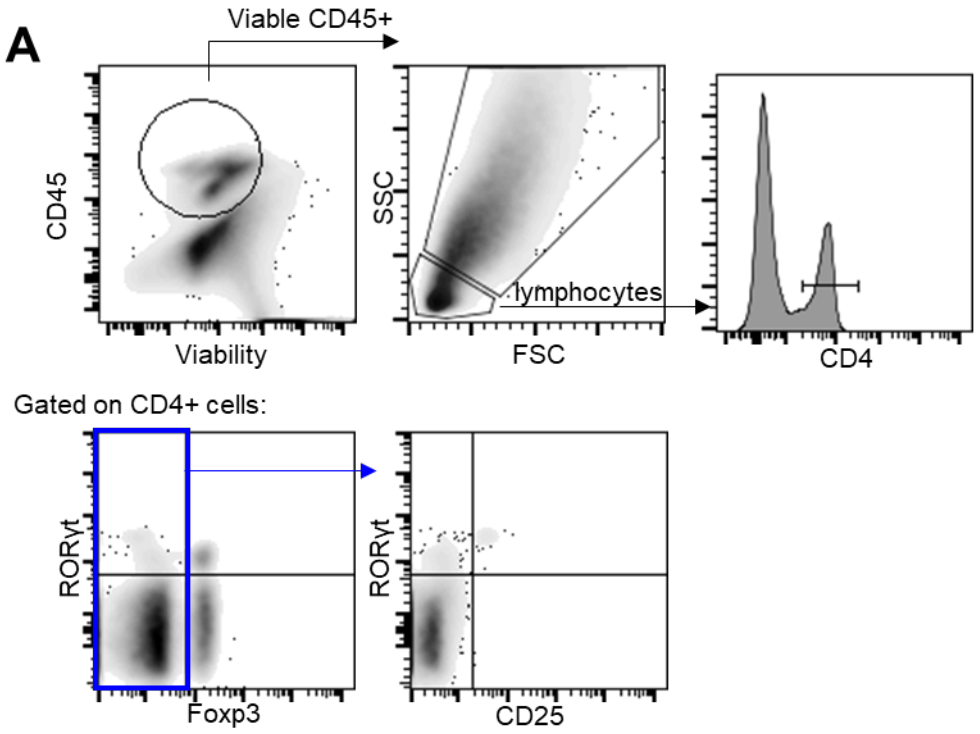
Kahalehili, et al. Front Immunol. 2021

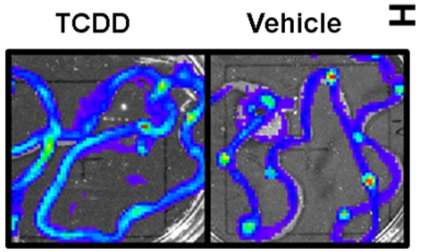
I3C promotes insulitis



Kahalehili, et al. Front Immunol. 2021

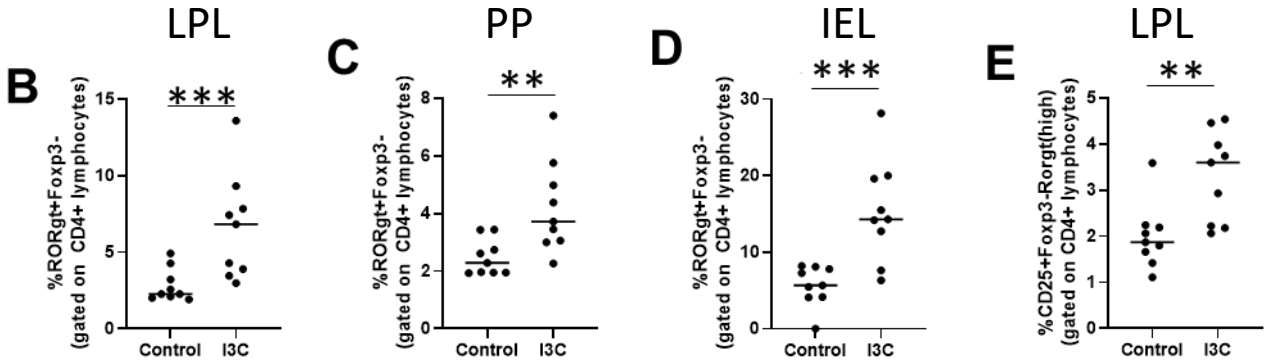
I3C increases intestinal Th17 cells!





Analyzed Th17, Tr1, Foxp3+ Treg populations in the LP, IEL, Peyer's patch, spleen, pancreatic lymph node

Tissue	Significant host factors	Control (avg)	I3C (avg)	Ratio (I3C: Control)	p value
IEL	RORgt+Foxp3-	5.64	15.38	2.73	***
IEL	CD4+Lag3+Foxp3-	3.83	8.48	2.22	**
LPL	high scatter CD4-IL10R+	36.36	24.86	0.68	***
LPL	RORgt+Foxp3-	2.85	6.63	2.33	***
LPL	CD25+Foxp3-RORgt^{high}	1.99	3.30	1.66	**
PP	Nrp1-Foxp3+	6.43	8.00	1.24	**
PP	RORgt+Foxp3-	2.48	4.22	1.71	**
PP	RORgt+Foxp3+	1.38	2.05	1.48	*
Spleen	CD4+IL-22+Foxp3-	0.64	0.87	1.37	**
Spleen	CD4+Nrp1highFoxp3-	2.54	2.13	0.84	*
Spleen	CD4+CD25+Foxp3-	2.00	1.56	0.78	*
Pancreas	Insulitis (>50)	26.56	39.08	1.47	*
Ileum	Ileum Cyp1a1	1.88	439.77	233.92	***



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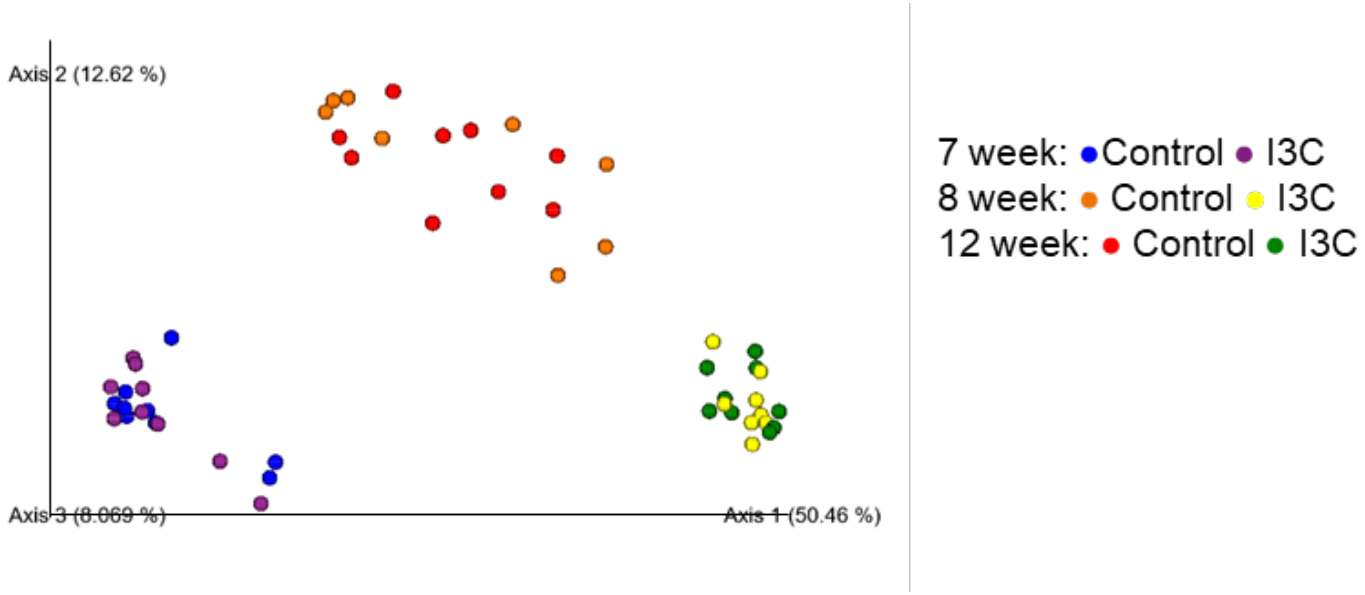
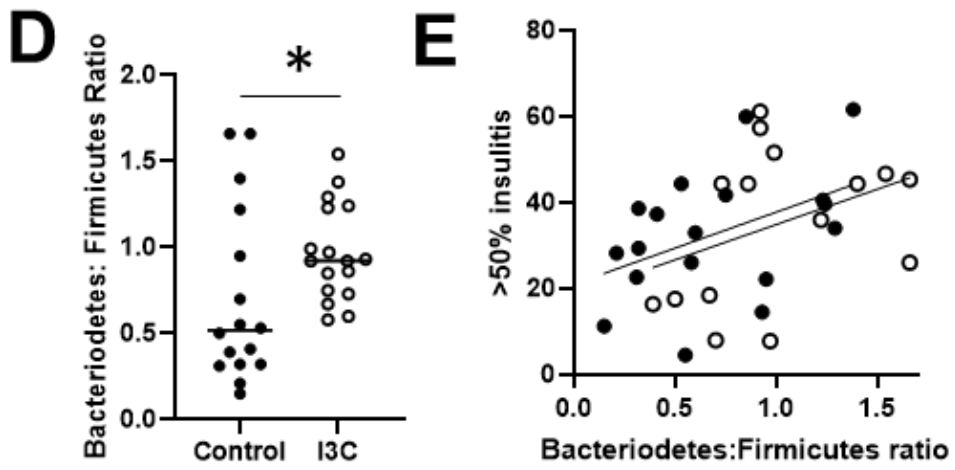
I3C alters gut microbial diversity





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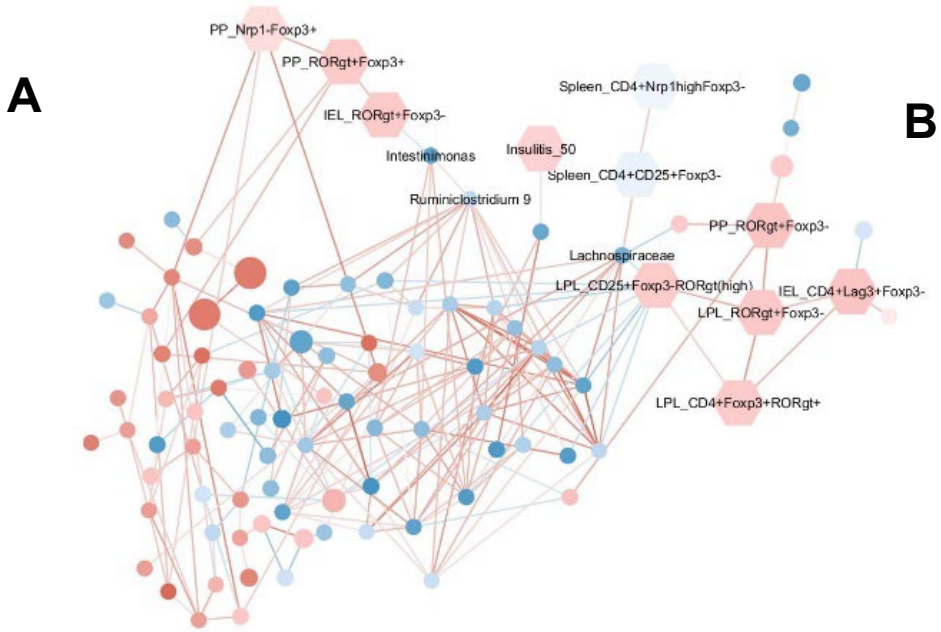
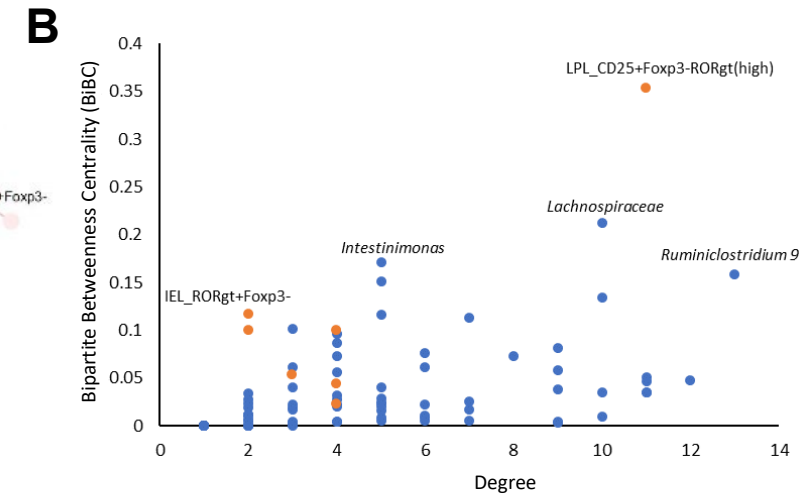
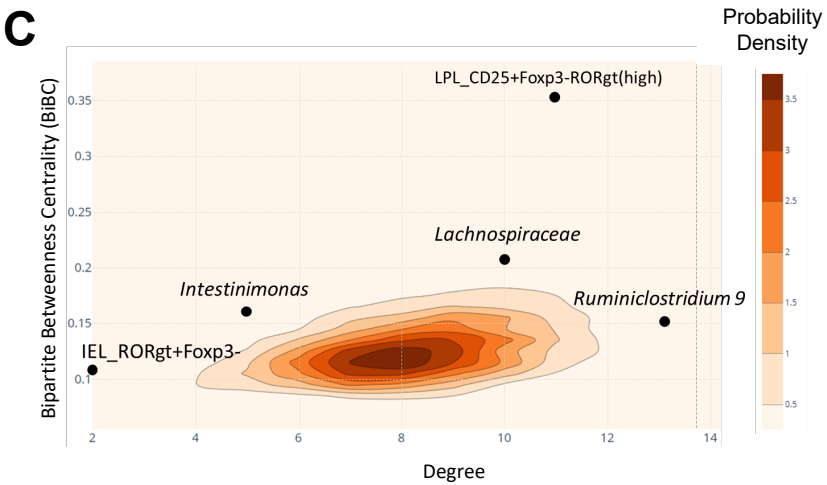
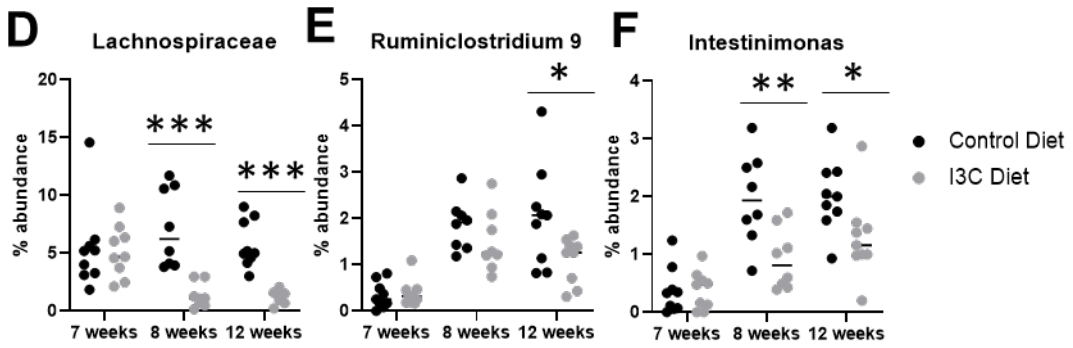
Transkingdom network predicts microbe-Th17 interactions

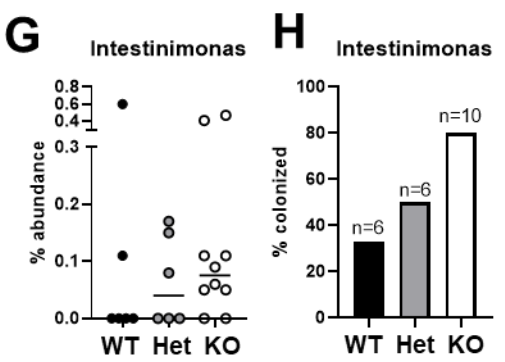






*Degree is the number of nodes each individual node interacts with, and measures the direct impact of one node has on other parameters in the system; bipartite betweenness centrality calculates the number of times the node lies in the shortest path connecting two groups of nodes, and more likely to be regulators of other nodes





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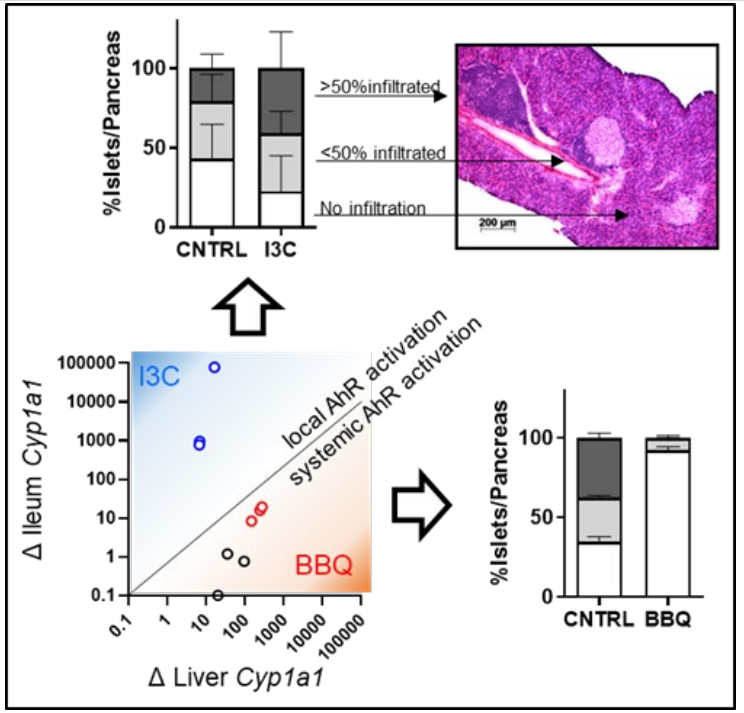
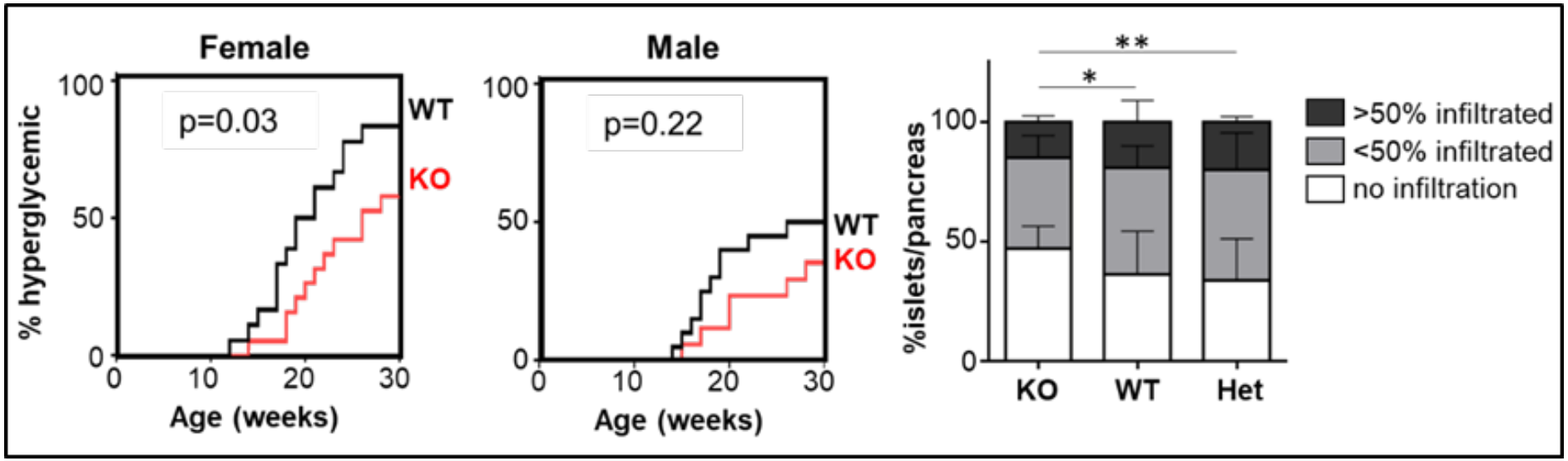
Speaking of AhR knockout mice…

- Strong systemic AhR activation (by TCDD and Cl-BBQ) prevents T1D development.
- Intestinal AhR activation (by dietary I3C) exacerbates insulitis.



- **How does physiologic expression of AhR impact T1D development?**



More questions than answers!

Why does I3C regulate immunopathology in C57BL/6 models, but not in NOD mice?

-opposite findings, disease exacerbation, no IL-22, decrease in butyrate-producing bacteria

-AhR allele sensitivity?

-background strain-specific differences?

-differences in IELs?

Why does systemic AhR activation and intestinal AhR activation lead to opposing Treg/Th17 ratios in NOD mice?

-differences in CD4+ T cell priming/microenvironment/role of microbiome?

Current studies using AhR knockout NOD mice (SPF and germ free) aim to answer the above questions!

Some final thoughts on AhR immunotoxicology…

- Both immune activation and immune suppression can be problematic
  - desired vs undesired effects: toxicology vs pharmacology
- Low levels of exposure to AhR ligands can be problematic depending on the context!
- CD4+ T cell differentiation is just one arm of AhR-immune interactions
  - B cells
  - CD8 cells
  - Developmental immunotoxicology
  - Myeloid cell development/function
  - ILCs
  - Gut homeostasis (IELs, IECs)
  - Microbiome-AhR interactions

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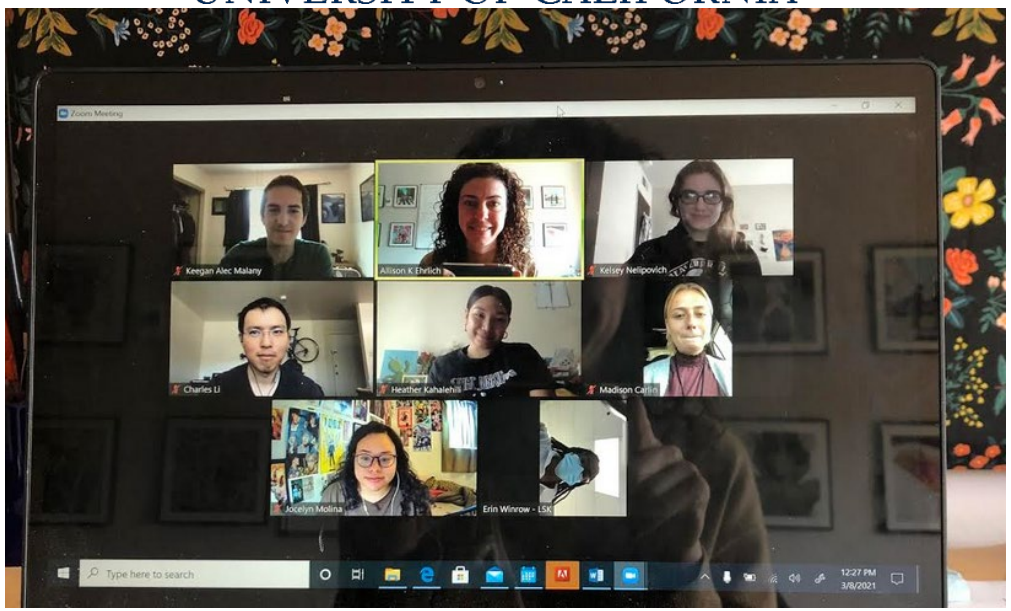

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