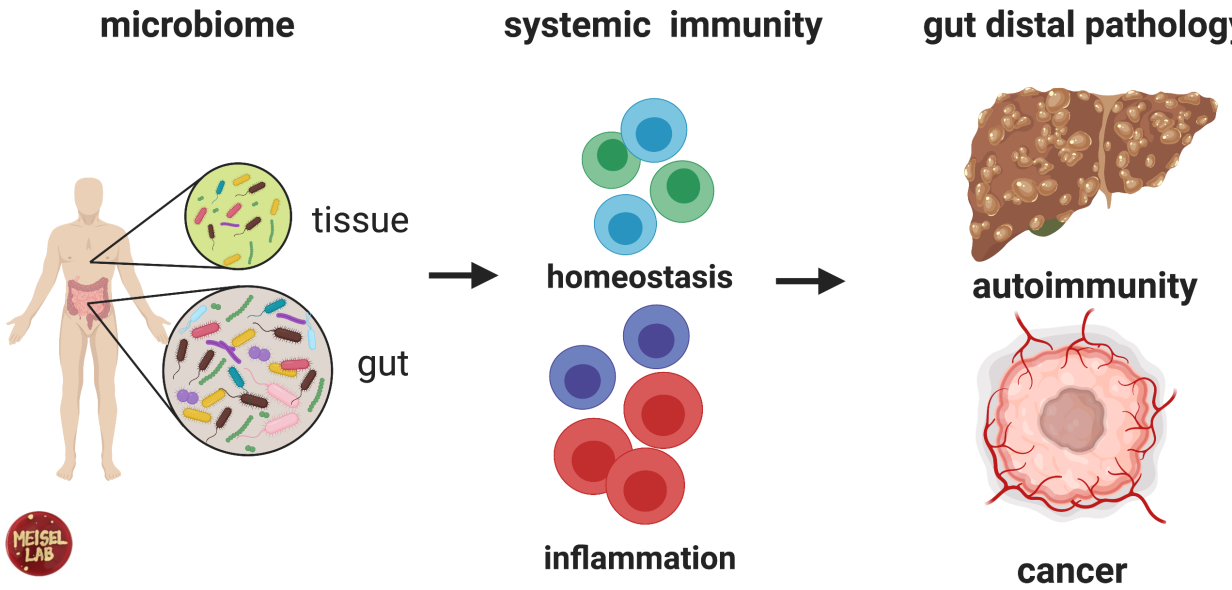
Exploring the role of the liver microbiome in autoimmune hepatitis

Tet2 deficiency drives liver microbiome dysbiosis triggering Tc1 cell autoimmune hepatitis



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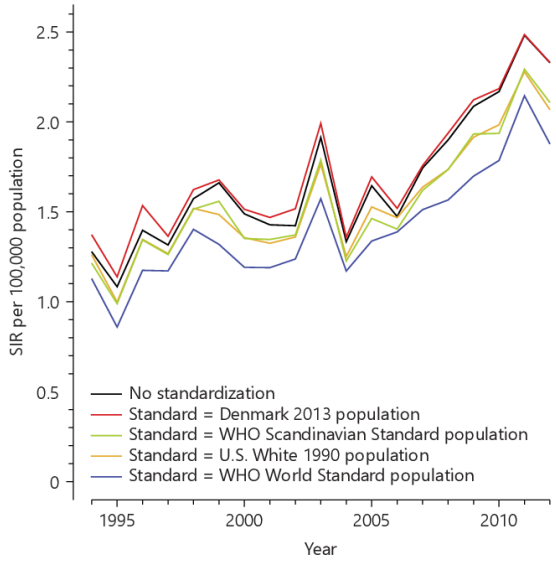
2024 PLRC Liver conference

MEISEL LAB

The drivers that trigger autoimmune hepatitis (AIH) remain to be defined

- T cell-mediated chronic, progressive, auto-inflammatory liver disorder
- genetic and environmental factors play a role, yet etiology unknown
- increasing incidence
- becomes refractory to immunosuppressants—the sole therapeutic option
- progresses to cirrhosis and end stage liver disease ->liver transplantation
- hepatic inflammation, mediated by IFNγ producing CD4 and CD8 T cells (Tc1 cells), sets the stage for overt AIH
  - Tc1 cells required in preclinical AIH



Jepsen et al., Dig Dis 2015

<u>Underlying mechanisms that triggers rampant hepatic Tc1 cell immunity that drives AIH remain undefined</u>

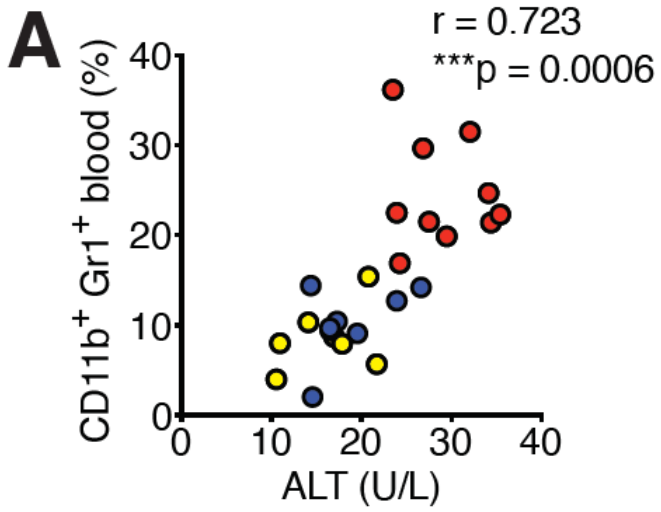
Manns et al., 2010; Ellis and Mann, 2012; 2005; Czaja et al., 2002; Ilyas et al., 2011; Bataller and Brenner 2005

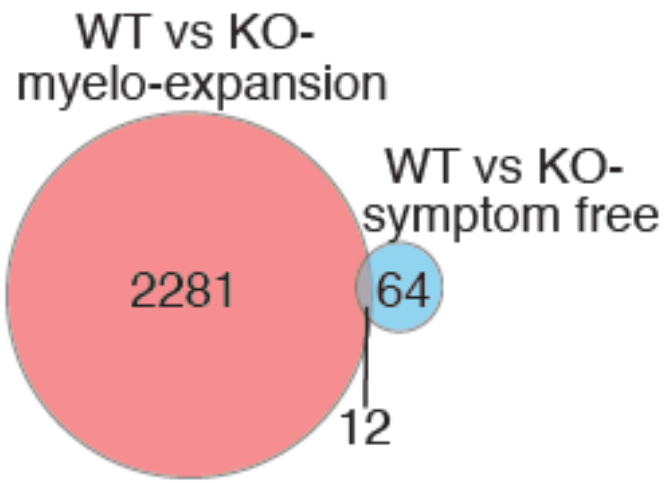
Hematopoietic *Tet methylcytosine dioxygenase 2* (*Tet2*) plays key role in autoimmunity

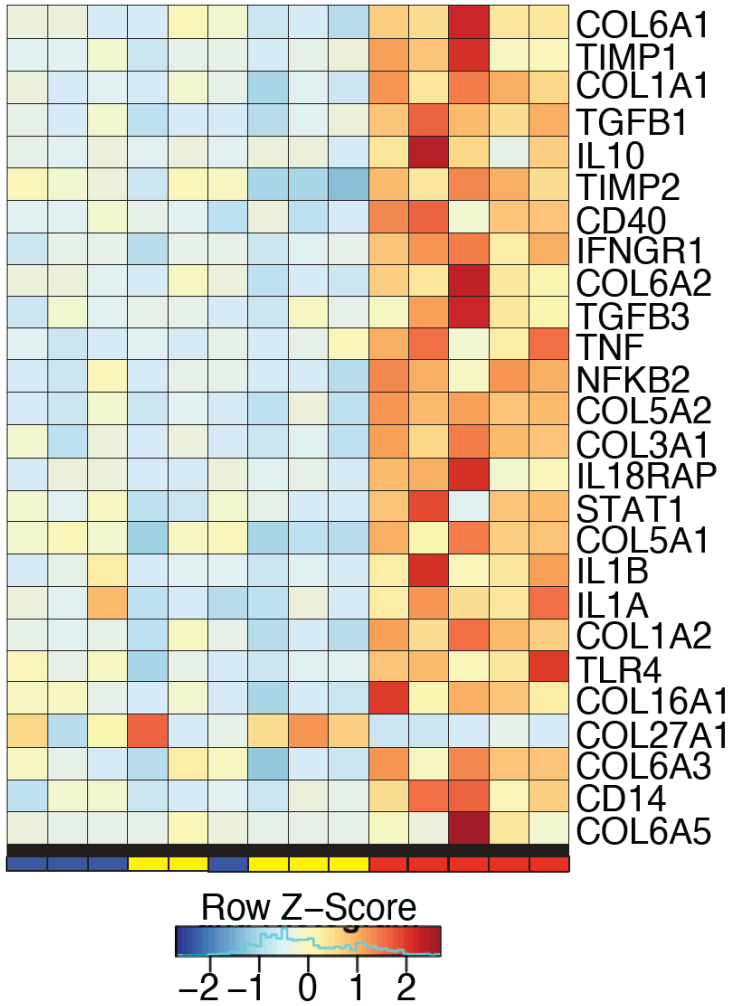
- *Tet2* key epigenetic regulator
- Somatic *TET2* loss of function mutations (~ 10% of healthy individuals > 50 years) increase the risk to develop cancer and autoimmune disorders, and chronic hepatic pathology [Wong et al., 2022 Nature]
- ~50-70% of (>20 wk old) *Tet2*^{VAV} mice develop CD11b^{+}Gr1^{+} myelo-expansion driven by systemic microbial signals [Meisel et al., Nature 2018]
- CD11b^{+}Gr1^{+} myelo-expansion shared pathological feature between *Tet2*-deficient mice/humans and patients with hepatic pathology

Is the expansion of circulatory myeloid cells in *Tet2*^{VAV} mice indicative of ongoing hepatic pathology?

Myelo-expansion in *Tet2*^{VAV} mice is indicative of ongoing hepatic pathology







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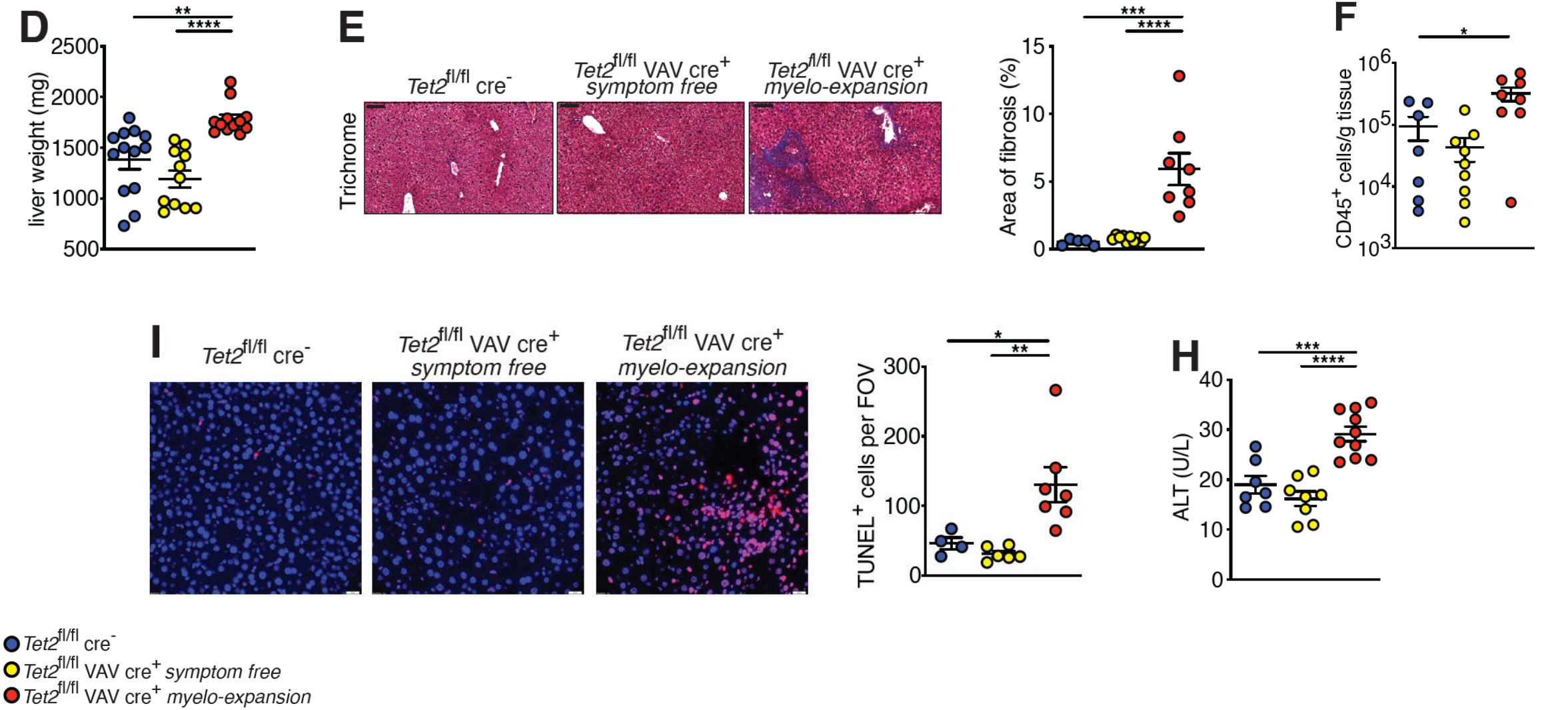
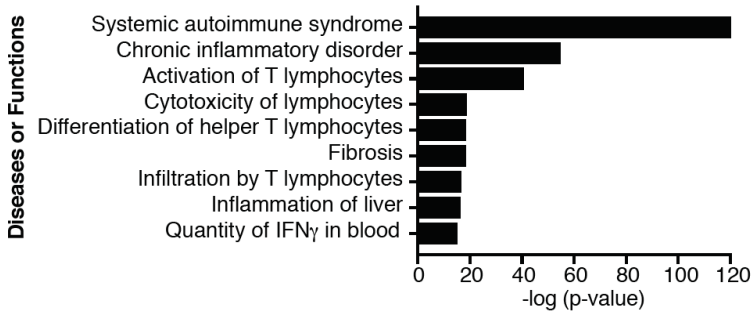
G. Arteel

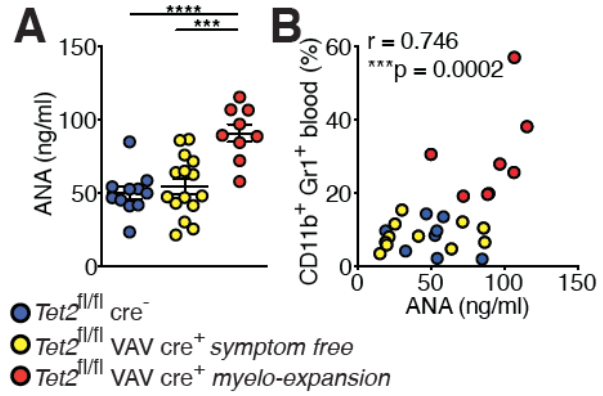
Tet2^{VAV} mice develop spontaneous hepatic pathology

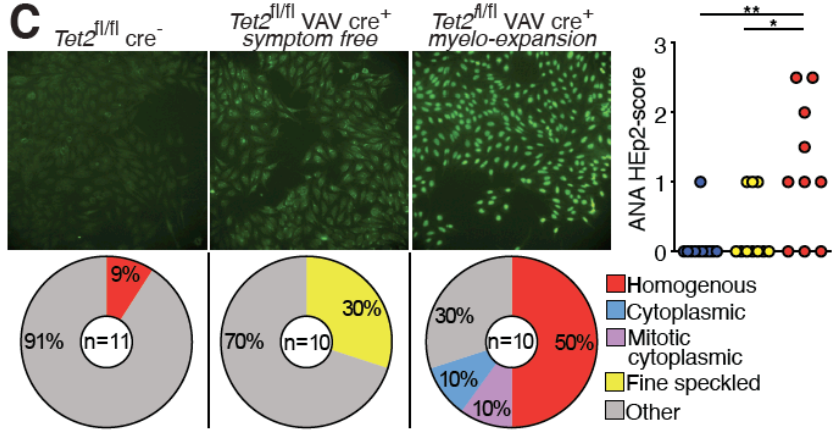


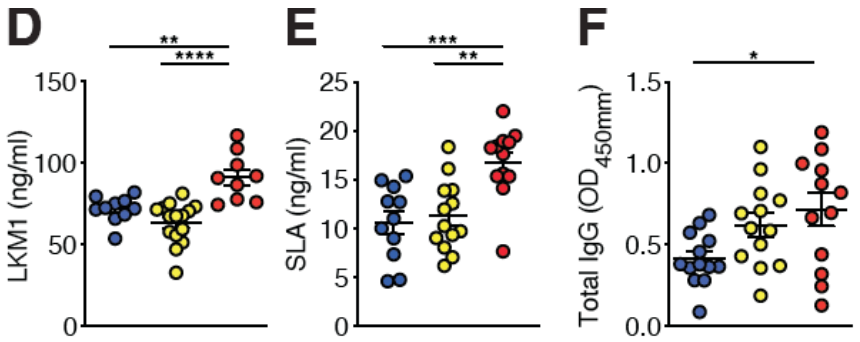
Hepatic disease in *Tet2*^{VAV} mice resembles AIH-like pathology

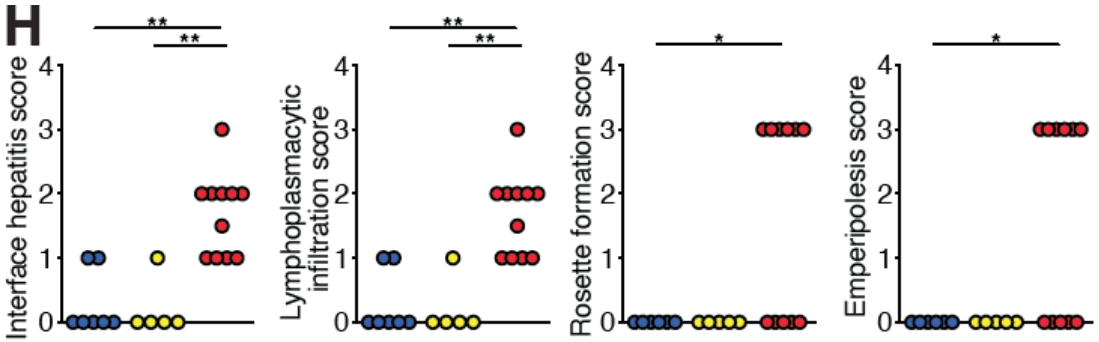
IPA analysis on liver RNAseq

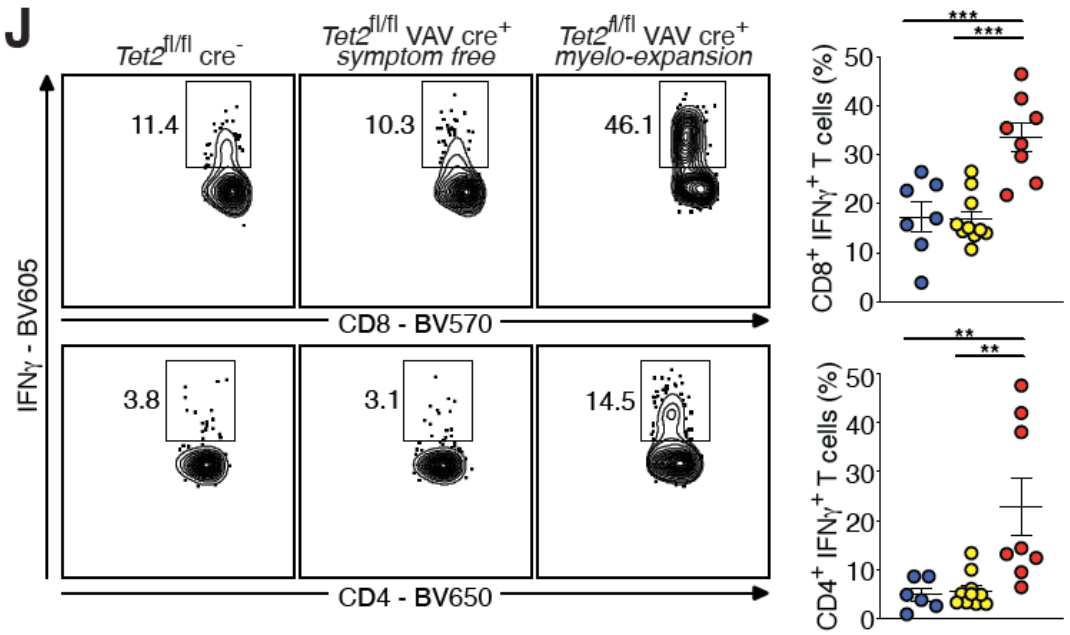












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A. Singhi

Interferon γ (IFNγ) plays an essential role in AIH

Correlation or causation?
Is IFNγ is a key driver of liver pathology in a *Tet2* deficient host?

-> IFNγ is a required driver of AIH-like pathology in a *Tet2* deficient host

Which cell type drives AIH-like pathology?

-> T cells are required, and deletion of *Tet2* in T cells is sufficient to drive AIH-like pathology

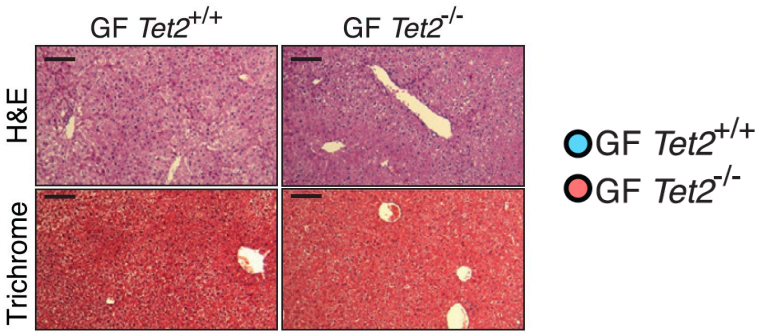
What are the triggers that drive IFNγ producing T cells in *Tet2*^{VAV} mice?

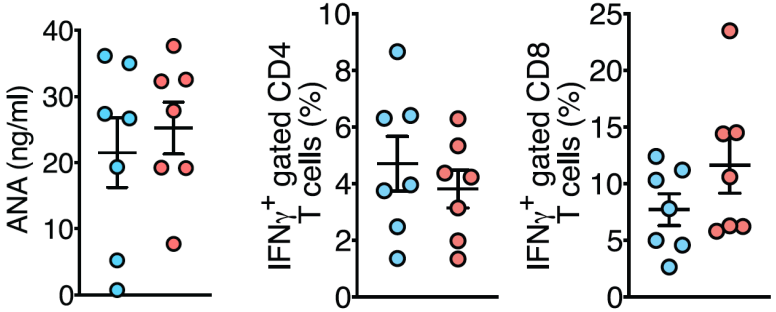
Gut microbiota plays a key role in liver disease development

Labs of Kriegel, Schnabl, Greten, Tilg & Elinav

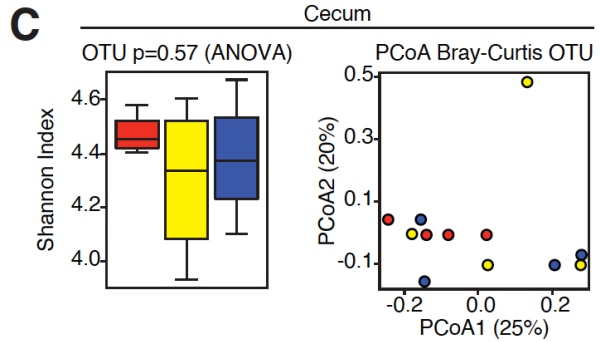
What is the role of the microbiota in the development of AIH in *Tet2*^{VAV} mice?

The microbiota is required and local liver dysbiosis is linked to AIH-like disease in Tet2^{VAV} mice

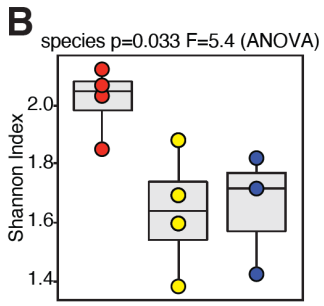




No gut dysbiosis

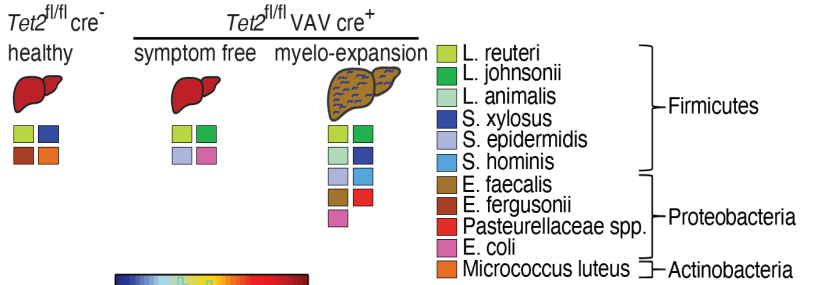


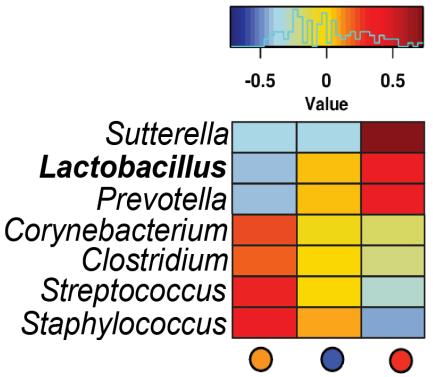
Liver 16S rRNAseq

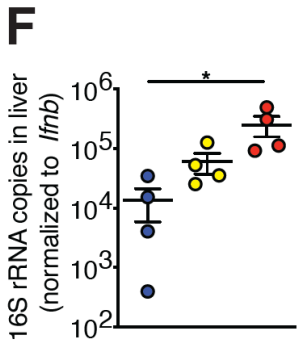


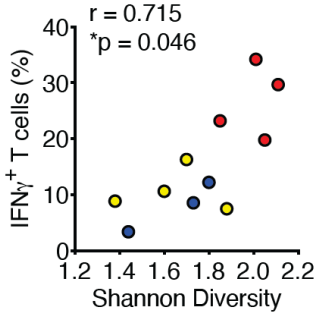
Labs of Kriegel, Miller & Schnabl: **The liver harbors a microbiome that may impact etiopathogenesis of hepatic disease**

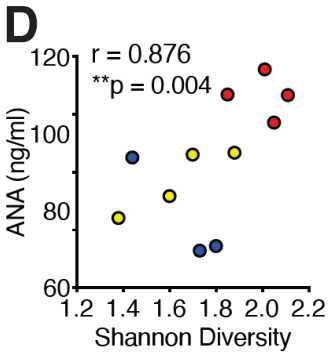
Liver bacterial culture







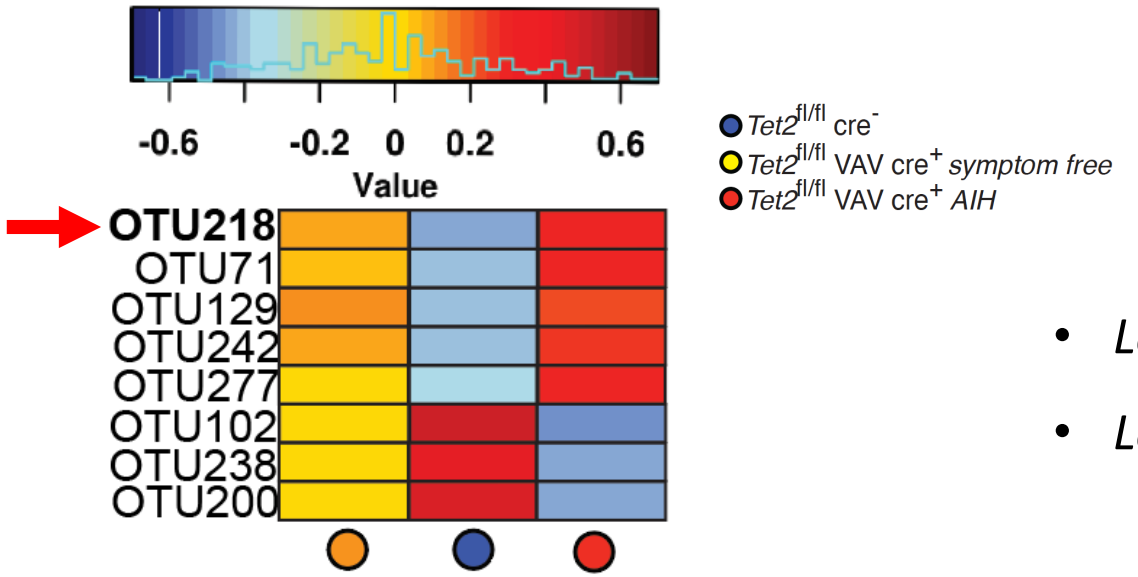




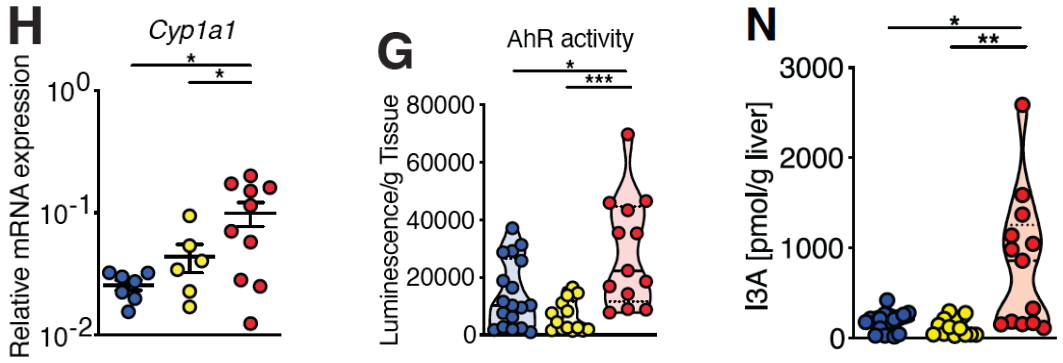
Tet2fl/fl cre-
Tet2fl/fl VAV cre+ *symptom free*
Tet2fl/fl VAV cre+ *AIH*

Liver dysbiosis in AIH-like *Tet2*^{VAV} mice is characterized by an enrichment of bacteria that can release aryl hydrocarbon receptor (AhR) ligands

Metagenomic profiling of metabolic microbial pathways



***Enrichment in microbial genes encode the Shikimate pathway*: Catabolize dietary tryptophan** -> aryl hydrocarbon receptor (AhR) ligands



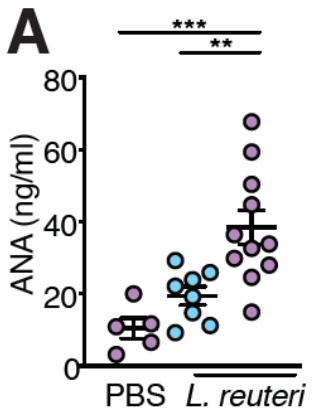
- *Lactobacillus spp.* increased in AIH *Tet2*$^{\Delta VAV}$ livers
- *Lactobacillus reuteri* highly potent AhR ligand producer
  - Primary AhR ligand indole-3-aldehyde (I3A)
- AhR agonists play a major role in T cell differentiation and effector function

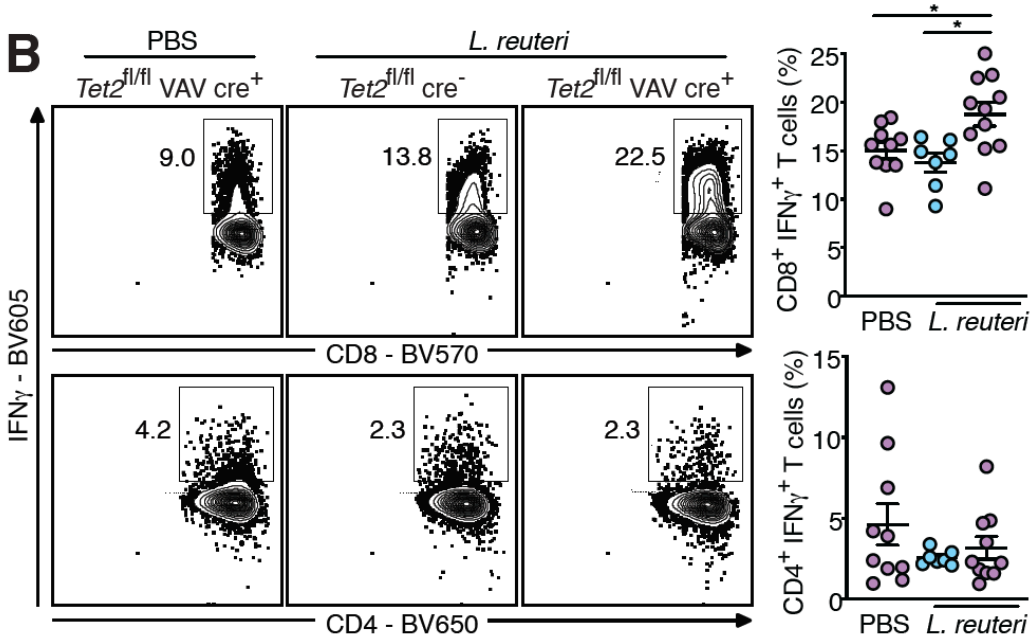
Does *L. reuteri* drive AIH-like disease by secretion of AhR ligands?

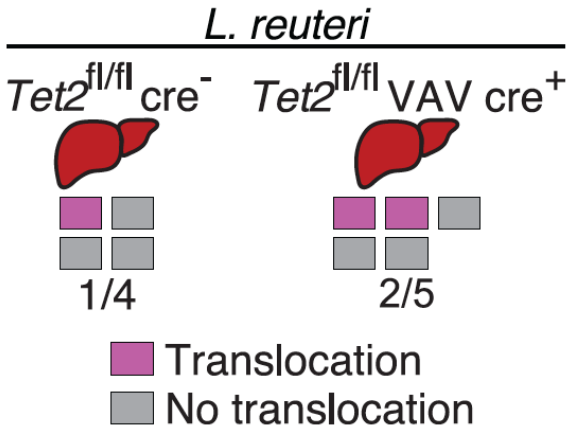
Hepatic translocation of AhR ligand releasing *L. reuteri* is sufficient to trigger AIH-like pathology in *Tet2*$^{\Delta VAV}$ mice

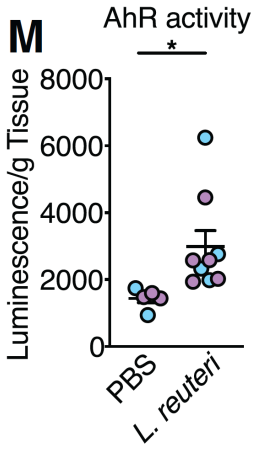
○ *Tet2*$^{fl/fl}$ cre^{-}
● *Tet2*$^{fl/fl}$ VAV cre^{+}

YOUNG MICE
(*no signs of AIH)

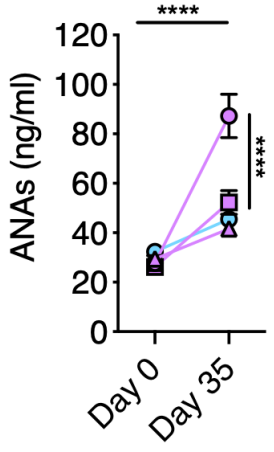






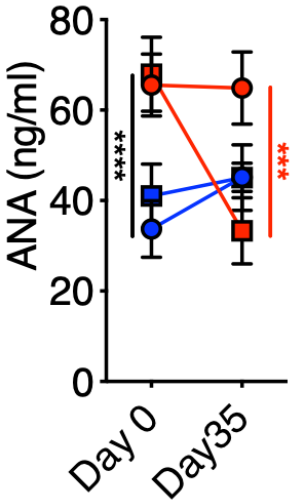


AhR activity is required to trigger AIH-like disease in *Tet2*$^{\Delta VAV}$ mice



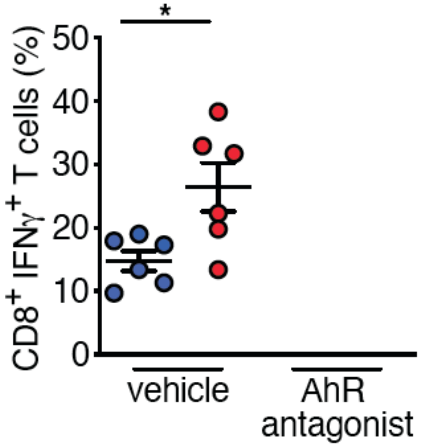
YOUNG MICE

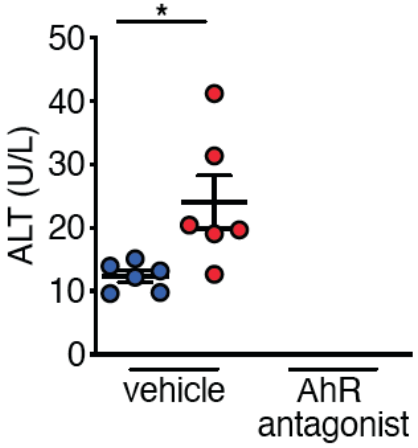
- *Tet2*$^{fl/fl}$ cre^{-} + *L. reuteri* + vehicle
- *Tet2*$^{fl/fl}$ VAV cre^{+} + PBS + vehicle
- *Tet2*$^{fl/fl}$ VAV cre^{+} + *L. reuteri* + vehicle
- *Tet2*$^{fl/fl}$ VAV cre^{+} + *L. reuteri* + AhR antagonist



> 20wk old mice

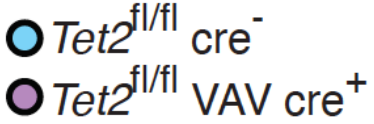
- *Tet2*$^{fl/fl}$ cre^{-} + vehicle
- *Tet2*$^{fl/fl}$ cre^{-} + AhR antagonist
- *Tet2*$^{fl/fl}$ VAV cre^{+} AIH + vehicle
- *Tet2*$^{fl/fl}$ VAV cre^{+} AIH + AhR antagonist

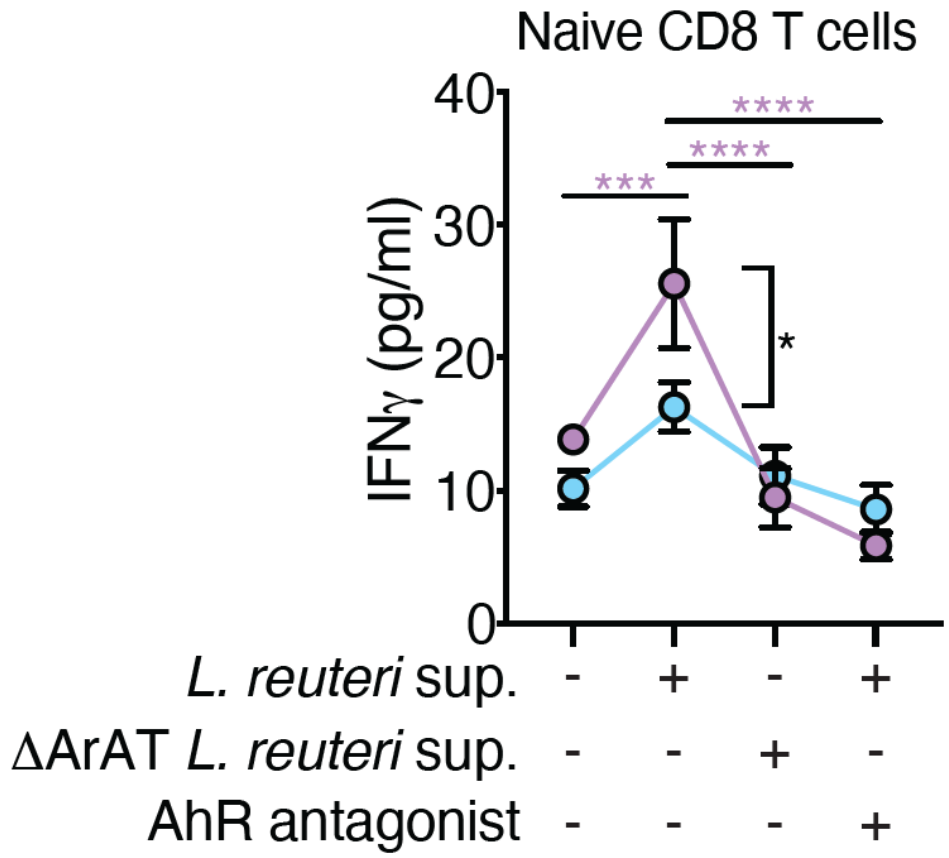




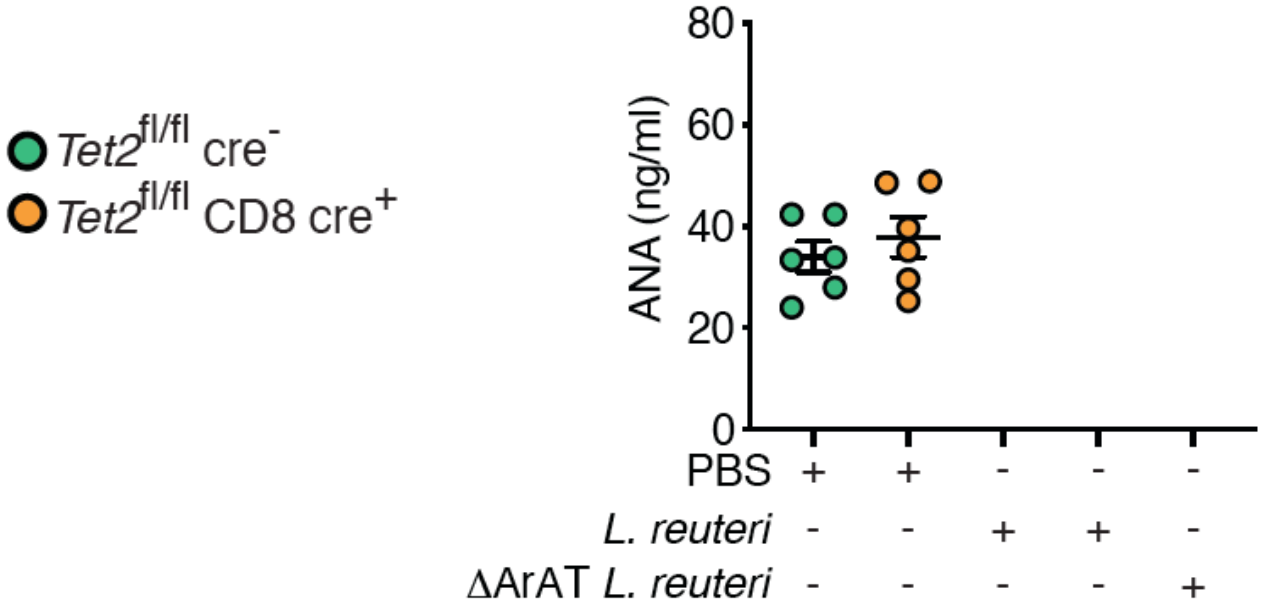
Tet2 in CD8 T cells antagonizes the selective induction of Tc1 effector function by *L. reuteri*-derived I3C in an AhR-dependent manner *in vitro*

ΔArAT *L. reuteri*
can not produce I3A
[Cervantes-Barragan et al., Science 2017]

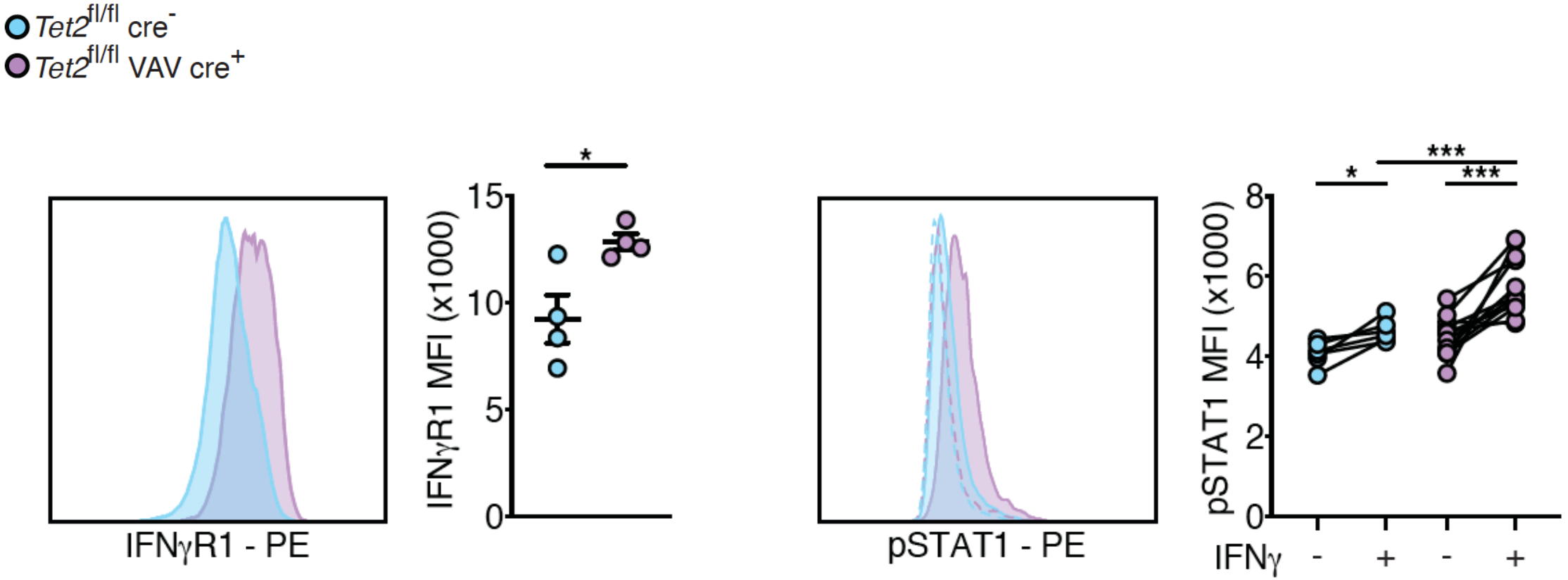




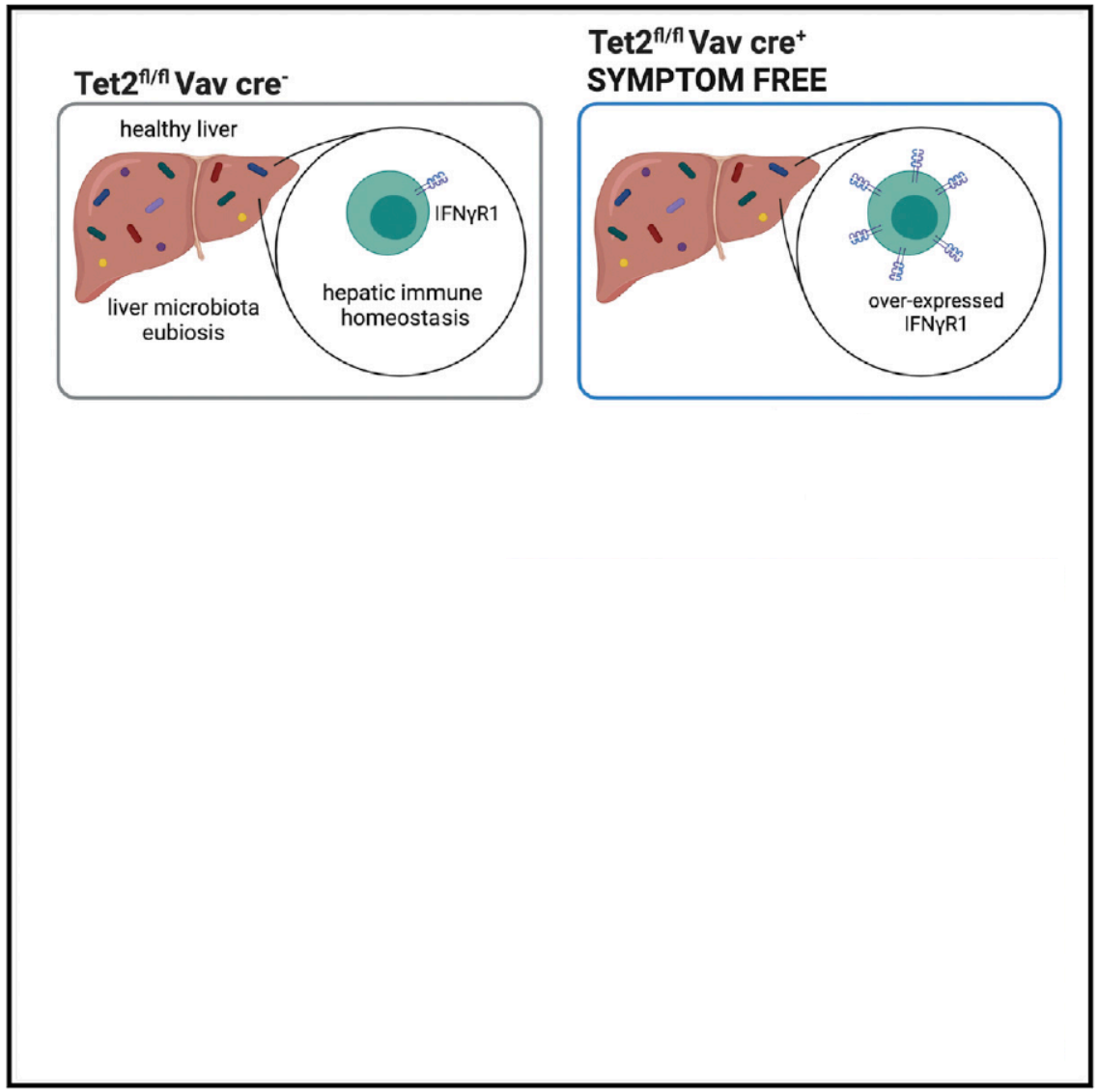
Tet2 in CD8 T cells antagonizes *L. reuteri*-derived I3A induced AIH-like pathology *in vivo*



Elevated expression of IFNγR1 naïve *Tet2*$^{-/-}$ CD8 T cells display enhanced sensitivity to extrinsic IFNγ leading to increased activation of STAT1 which maintains a type 1 immune effector program



Summary & Future perspective



- What drives liver microbiome dysbiosis in our model?
- What is the role of the liver microbiome/ AhR ligand producing commensals in other AIH models?
- Role of AhR ligand producing bacteria in human AIH?

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