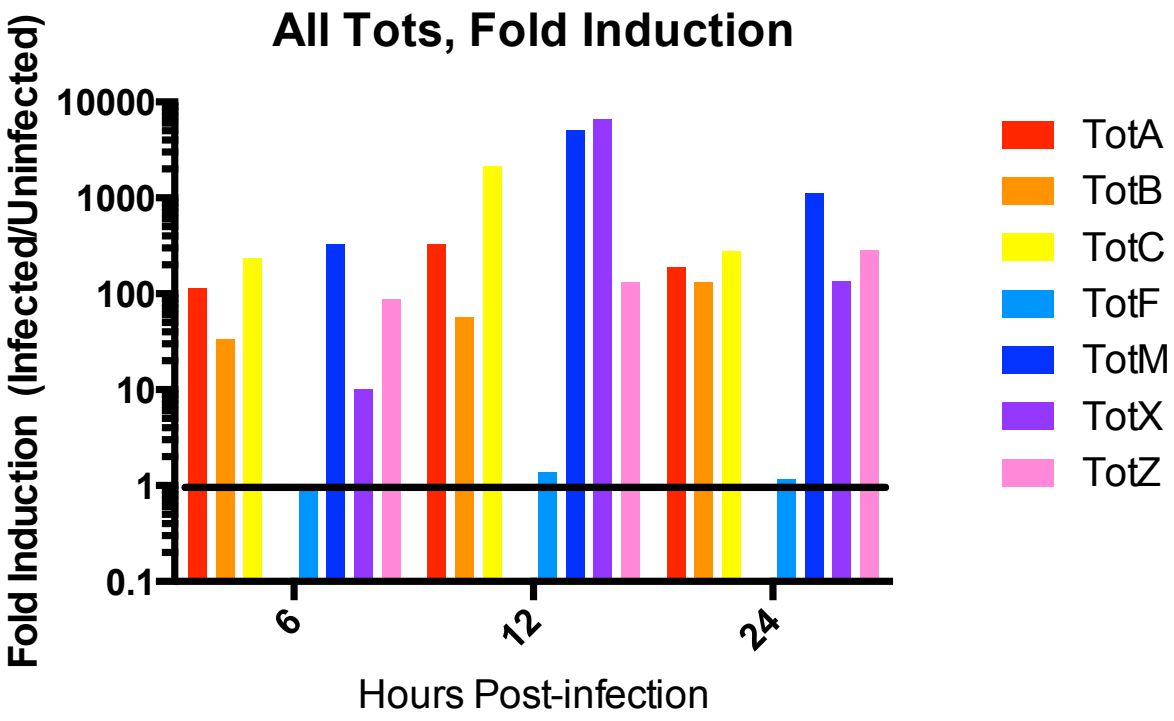
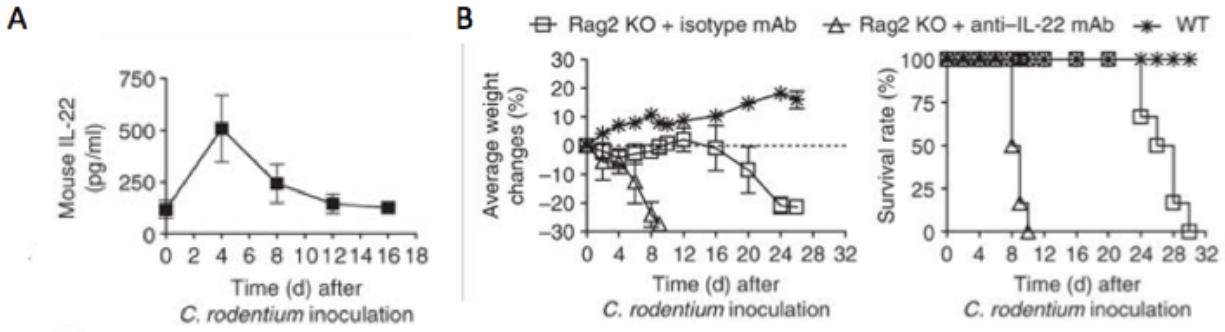
1. You are studying the *Drosophila* response to DNA virus infection, using Irido Iridescent Virus 6 (IIV6) as a model. You find that IIV6 robustly induces the expression of several Turandot (Tot) genes in cells and animals. Note, the eight Tot genes encode 8 homologous small proteins that are induced by various infectious and stress challenges, but have poorly characterized function(s)



Legend: Following IIV6 infection wild-type flies induced expression of Turandots A, B, C, M, X, Z. Turandot F was uninduced. Turandot E was not detected

Provide a hypothesis for the mechanism of IIV6-triggered Tot gene expression, and provide 2 Specific Aims that will examine this hypothesis.

2. The transcription factor RORγt is critical in the development of inflammatory T effector Th17 cells as well as other cell types, that contribute to immune responses against bacterial and fungal infection. Indeed, mice deficient of RORγt expression is susceptible to *Citrobacter rodentium* infection. RORγt is known to induce various cytokines including IL-17 and IL-22. After *C. rodentium* inoculation, however, both IL-17 receptor knockout mice and WT littermates survived infection without any significant weight loss or any differences in colon histology. While IL-17 protein level is detected in 7~9 days after bacterial infection, IL-22 is strongly induced markedly earlier, at 4 days, as shown in panel A. RAG2 KO mice, which lack T and B cells, showed more severe weight loss compared to WT control upon infection, but treating RAG animals with anti-IL-22 further exacerbated colitis phenotypes, as seen in panels B.



Propose a hypothesis explaining these findings and provide two Specific Aims to test it.

3. It is established that nucleic sensing endosomal TLRs contribute to systemic autoimmune diseases such as SLE. TLR9-deficient autoimmune prone mice fail to make autoantibodies reactive to dsDNA or chromatin, and TLR7-deficient mice fail to make autoantibodies reactive to RNA or RNA-associated proteins. Moreover, in every murine model of SLE evaluated to date, mice with defects in both TLR7 and TLR9, or simply TLR7, have markedly reduced autoantibody titers, immune activation, and end-organ disease. However, paradoxically, TLR9-deficiency alone results in more severe clinical disease. To better understand the connection between TLR9-/- antigen presenting cells and T cell activation, WT and TLR9-/- mice that express an ovalbumin in MHCII-expressing antigen presenting cells (IiTGO mice) were used. This transgene is activated by feeding doxycycline, providing a highly controlled experimental system. These mice can then be injected with WT ovalbumin specific T cells and monitored for disease manifestations.

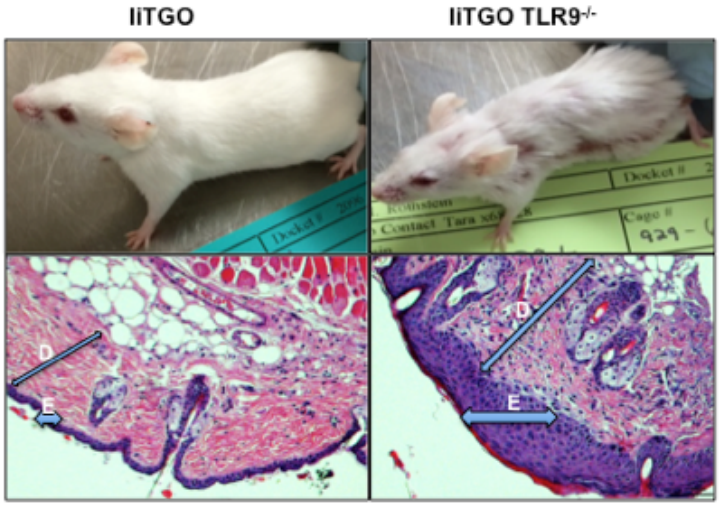


Fig. 1. Loss of TLR9 in IiTGO mice causes cutaneous SLE. Mice and skin histology of IiTGO WT and TLR9^{KO} mice 4 wks post T cell injection. E = Epidermis and D= Dermis.

Only the TLR9-/- recipient mice develop "cutaneous lupus" (Fig. 1), associated with the increased migration of migration of OVA-specific T cells into the skin. Comparable numbers of OVA-specific T-cells cells can be isolated from the spleen, but when these cells are stimulated with anti-CD3, only T cells isolated from the TLR9-/-, ovalbulmin-expressing cells make the pro-inflammatory cytokine IL-17 (Fig. 2). B cells from the TLR9-/- mice are more activated. They have more germinal center cells and more antibody producing cells (Fig. 3).

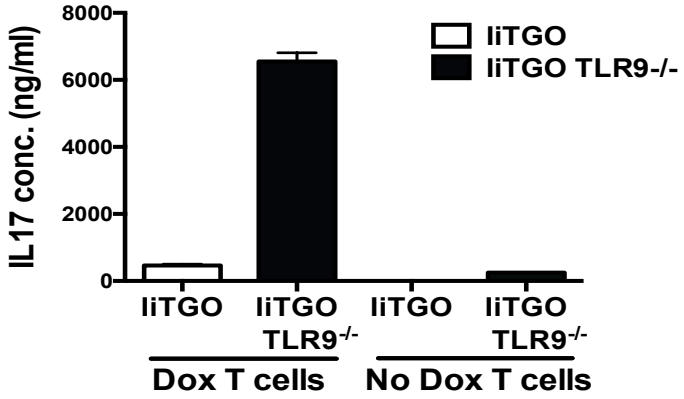


Fig. 2. DO11 T cells injected into IiTGO TLR9-/- mice produce high levels of IL-17. *KJ126-purified DO11 cells isolated from IiTGO WT and IiTGO TLR9^{KO} mice were activated on anti-CD3 coated plates for 24h. Supernatants were assayed for IL-17 by ELISA.*

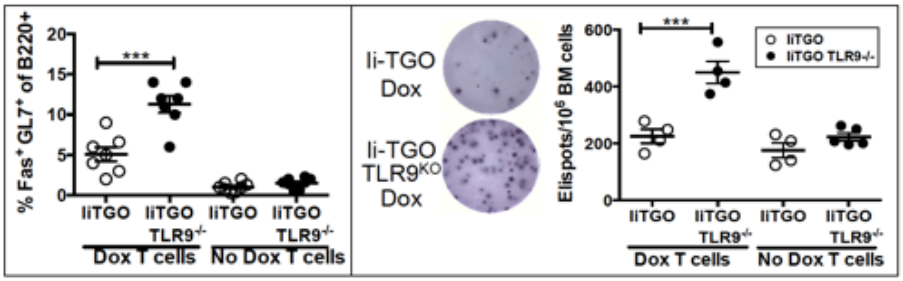


Fig. 3. B cell activation in IiTGO mice. *IiTGO WT and TLR9^{KO} mice were injected with DO11 T cells and analyzed for (A) germinal center B cells in the spleen by flow cytometry and (B) plasma cells in the BM by ELISPOT assay.*

Propose a hypothesis to explain how TLR9-deficiency leads to these outcomes, as well as 2 Specific Aims to address this hypothesis.