Blocking Anti-TLR3 mAb for Inflammation

Y.Morel

Molecular Pattern Recognition Receptors

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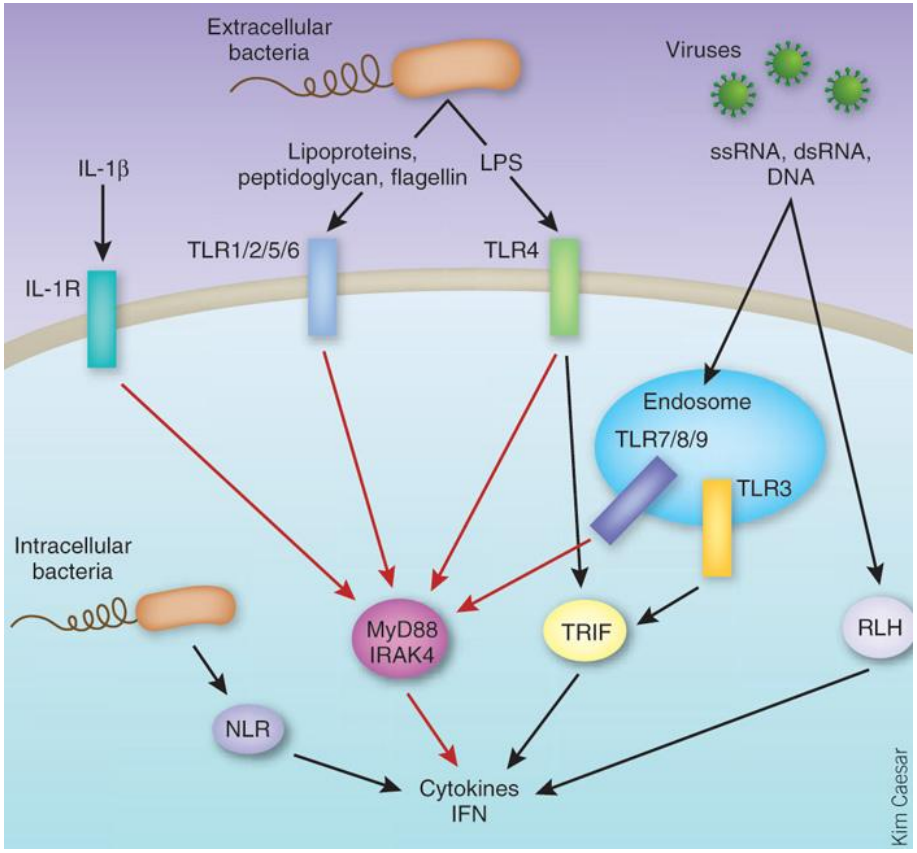
Boston, MA

innate pharma

TLR Family

- Toll-like receptors (TLR) are a family comprising 10 members in human and are part of **Pattern Recognition Receptors** used by the innate immune system
- Receptors for bacterial components
  - TLR2: lipoprotein, peptidoglycan
  - TLR5: flagellin
  - TLR4: LPS, MPL
  - TLR1 and TLR6: TLR2 co-receptors
- Receptors for viral components
  - **TLR3: dsRNA**
  - TLR7/8: ssRNA
  - TLR9: CpG motif in ssDNA
  - RLH: 3P-ssRNA, dsRNA

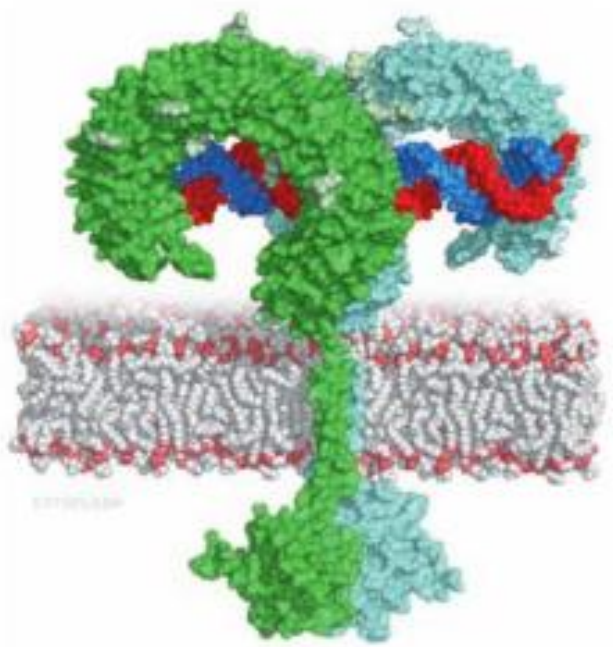


TLR3 : An Endosomal Inflammatory Receptor for dsRNA

- TLR3 is mainly localized in the **endosomes-lysosomes**, but it can be detected at **cell surface**

- TLR3 recognizes **viral dsRNA**, but also **U1small nuclear RNA** and **mRNA**, potentially originating from **necrotic cells**

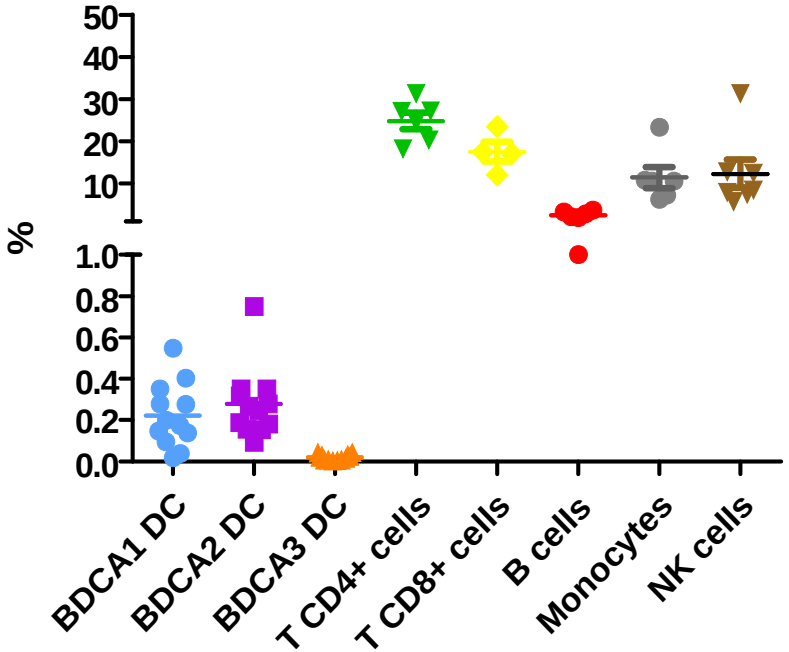
- TLR3 stimulation induces
  - strong **type I IFN**
  - **pro-inflammatory cytokines/chemokines**
    - IL-6, RANTES , MCP-1, MIP-1α, IL-1β, TSLP, IL-17
  - DC activation, monocyte and epithelial cell differentiation



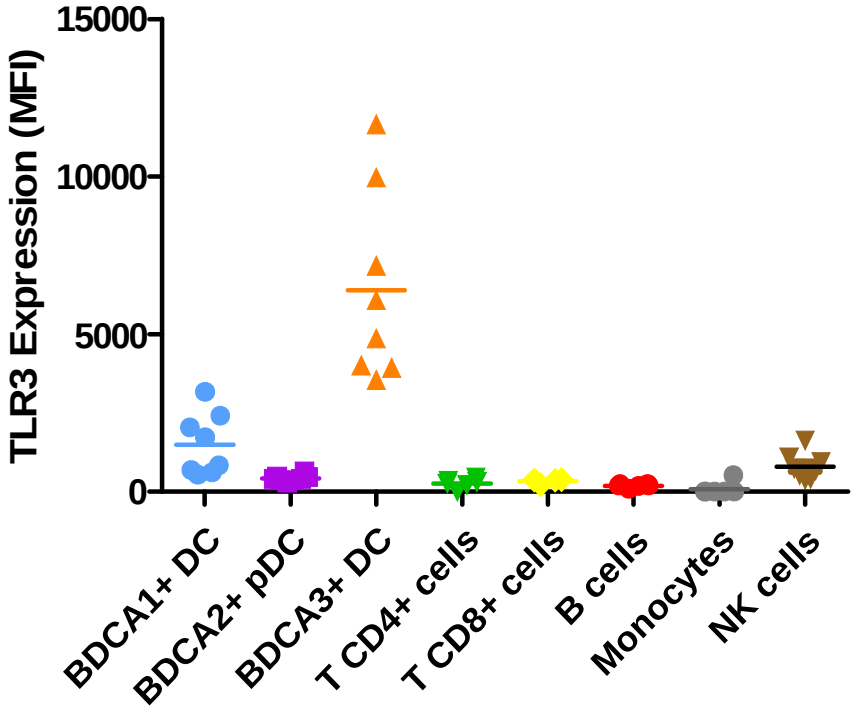
Liu et al, Science. 2008

TLR3 Expression is Highly Restricted to BDCA3+ DC among Human PBMC

PBMC subsets

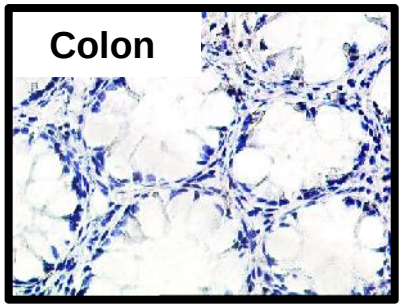


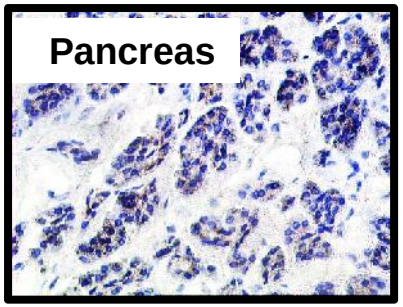
TLR3 expression

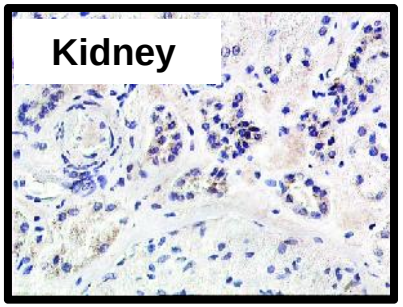


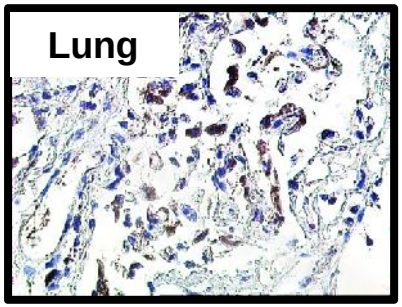
- No TLR3 expression on human neutrophils
- Performed with proprietary specific anti-TLR3-PE mAb

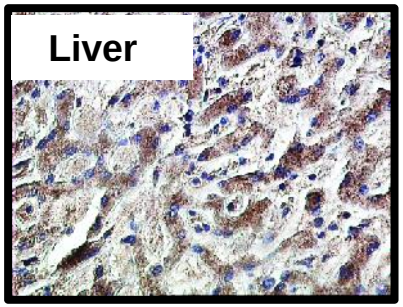
TLR3 is Expressed on Epithelial Cells in Human Normal Tissues

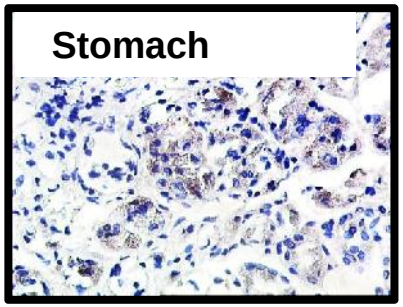


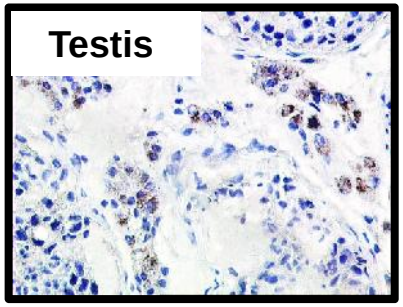


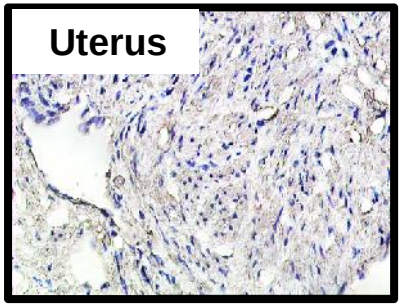


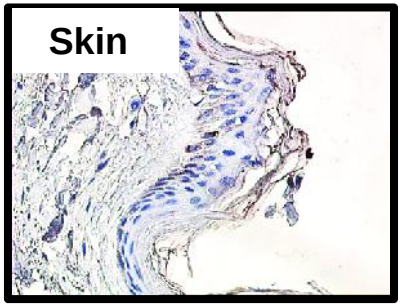








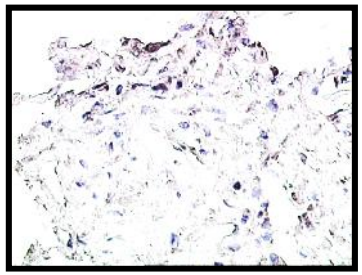




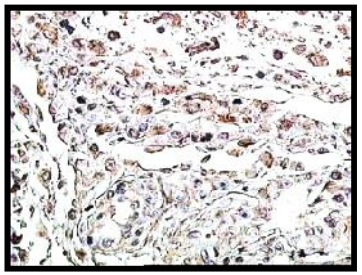
Performed with proprietary specific anti-TLR3 mAb on FFPE tissue

TLR3 is Over-Expressed in Human Inflammatory Tissue

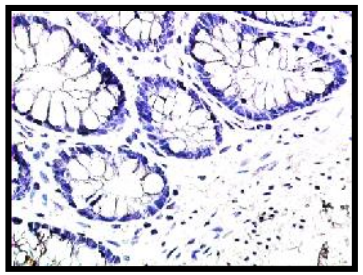
Normal knee



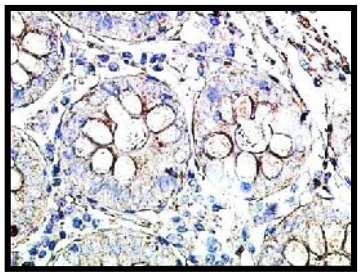
RA knee



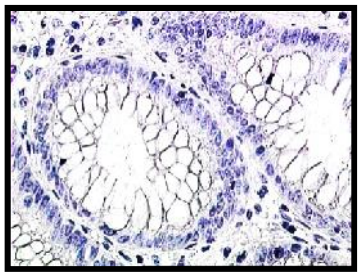
Normal colon



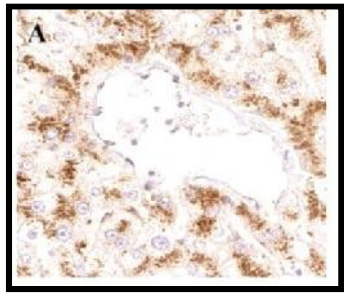
Crohn Disease



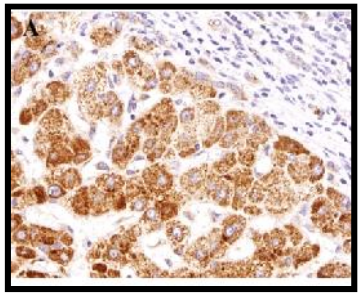
Ulcerative colitis



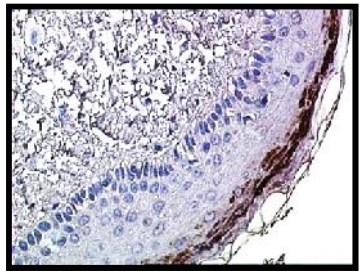
Normal Liver



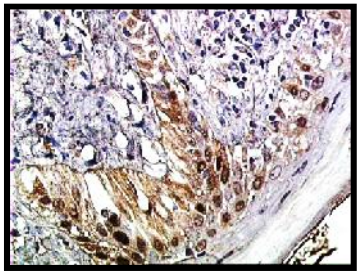
Cirrhotic Liver (HCC but not tumor cells)



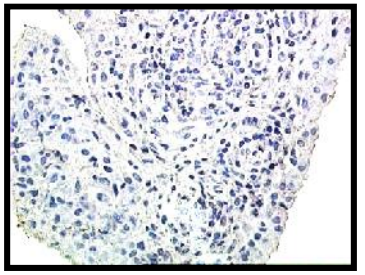
Normal Dermis



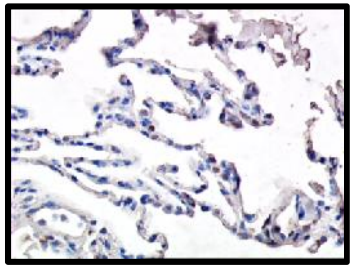
Lupus erythematosus



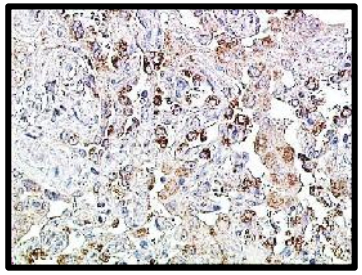
Psoriasis



Normal Lung

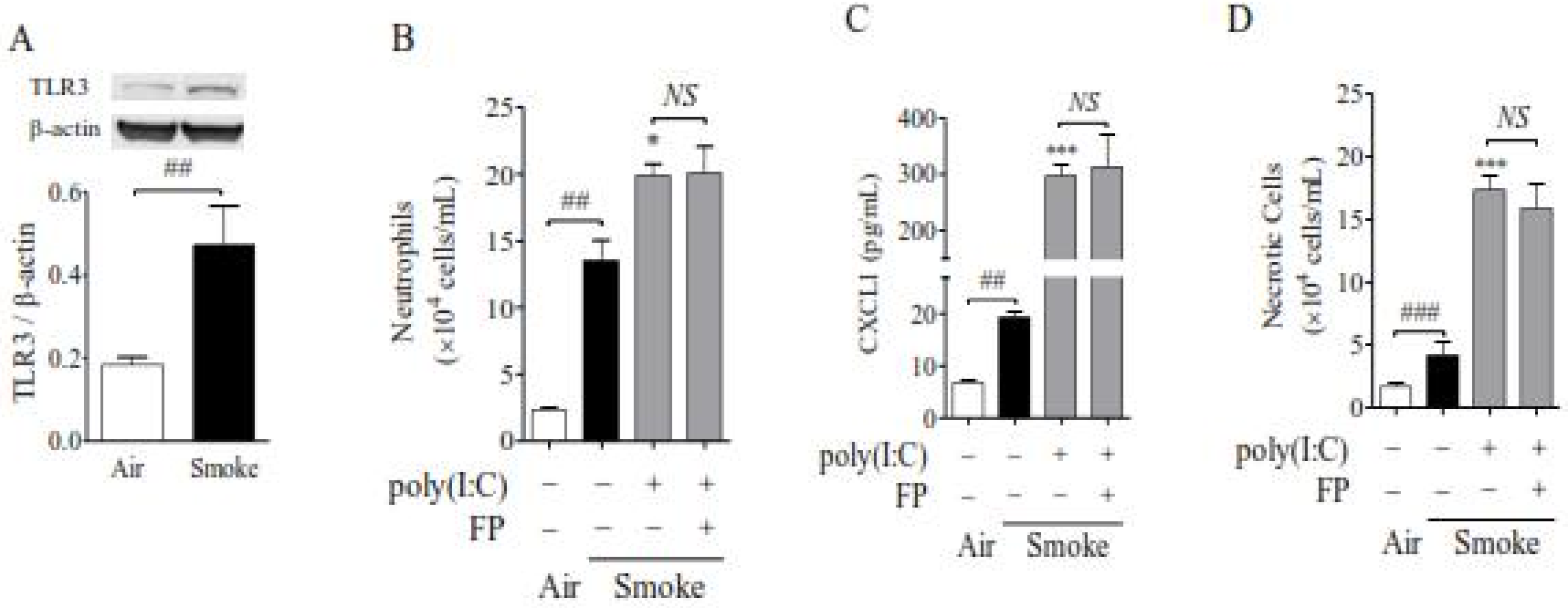


Sarcoidosis



Performed with proprietary specific anti-TLR3 mAb on FFPE tissue

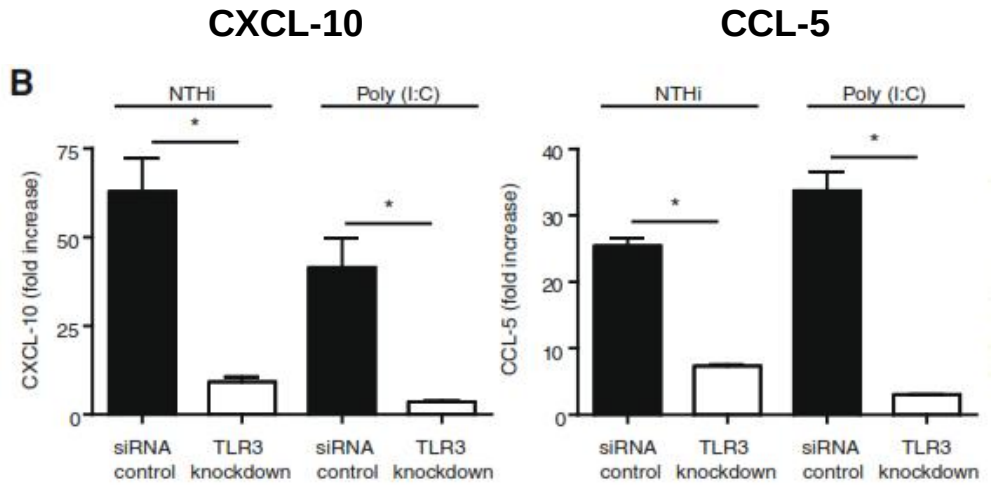
TLR3 Expression and Response in Cigarette Smoke-induced Airway Inflammation Models in Mice



Kimura et al, Chest. 2013

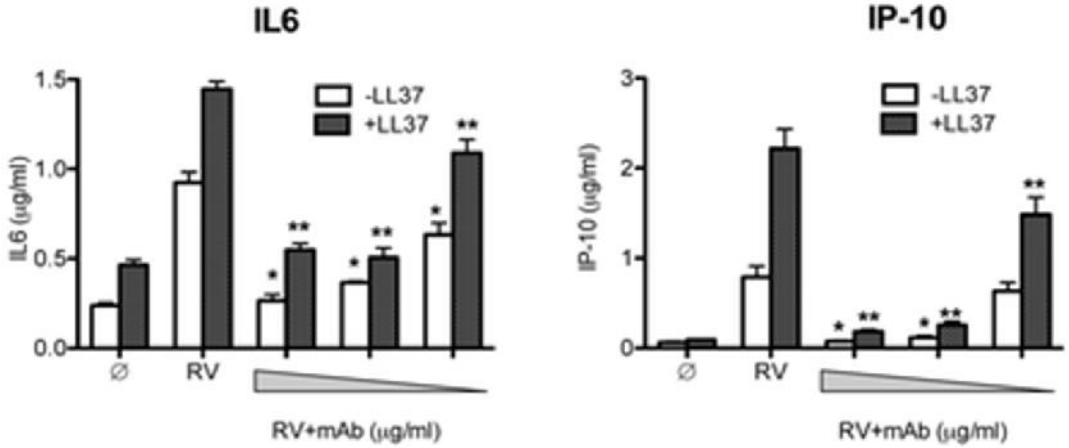
TLR3 Mediates Inflammatory Cytokine Response to Infections in Airway Epithelial Cells

- Non Typeable Haemophilus influenzae



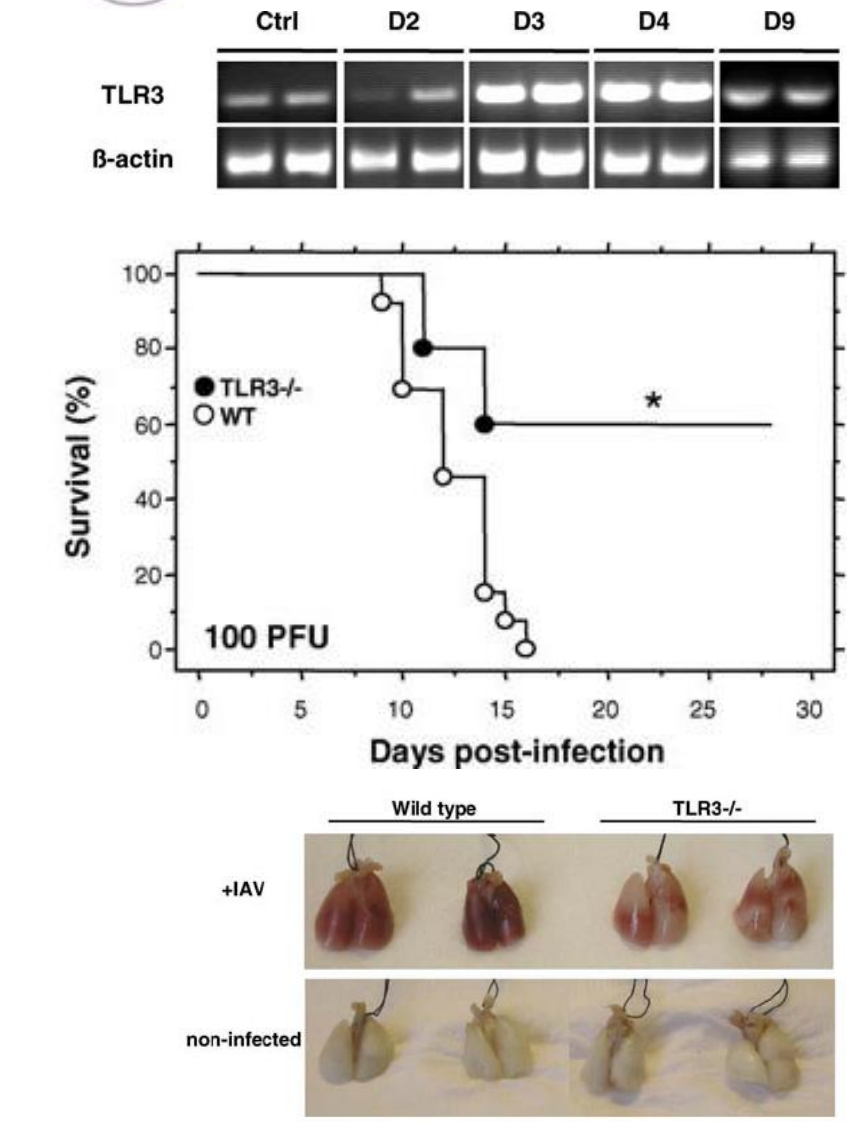
Teng et al, Cell Immunol. 2010

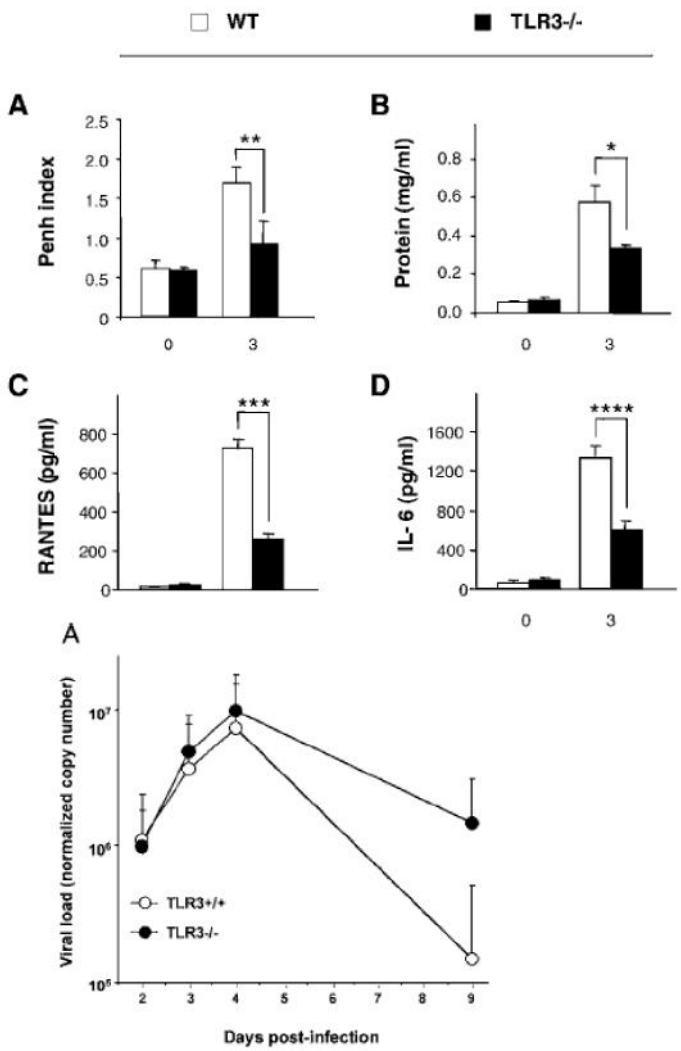
- Rhinovirus (RV)



Lai et al, PLoS ONE. 2011

TLR3ko Mice are Protected from Lethal Inflammatory Pneumonia in Influenza Virus infected Mice

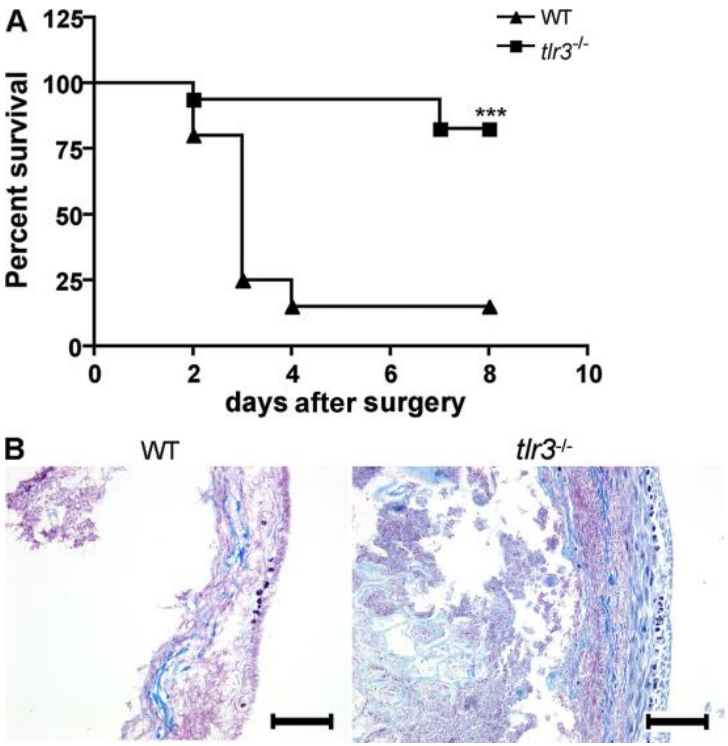


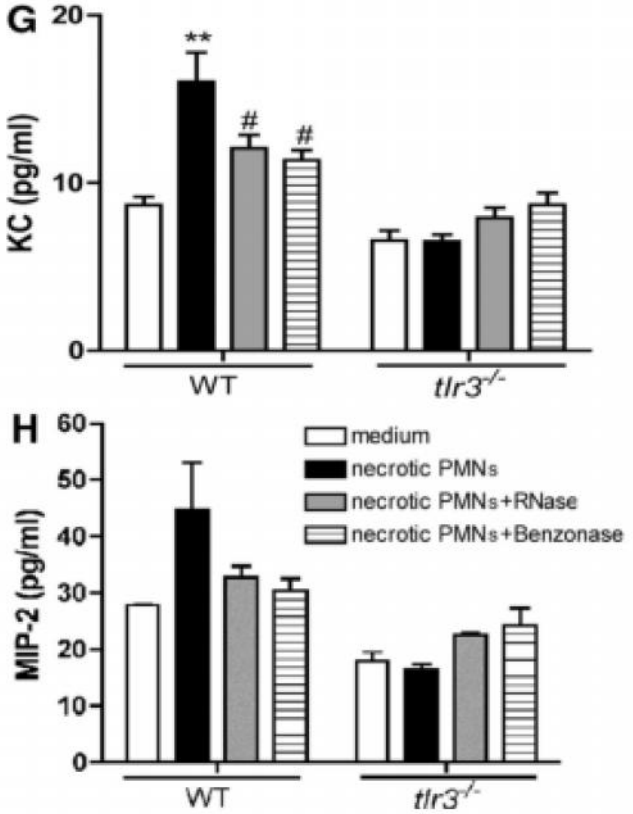


Le Goffic et al, Plos Pathogen. 2006

Blocking TLR3 Protects Mice in Sepsis and Gut Ischemia Models

TLR3ko and anti-TLR3 pAb treated mice are resistant to lethality and cardiac dysfunction after CLP surgery and exhibit less tissue damage and late inflammation after gut ischemia

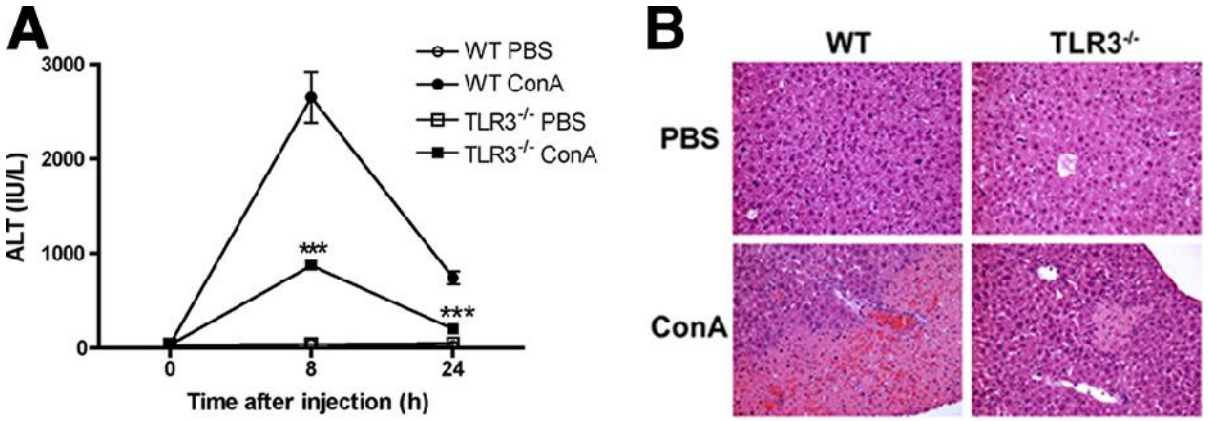




Cavassani et al, J Exp Med. 2008
Ming et al, Crit Care Med. 2012

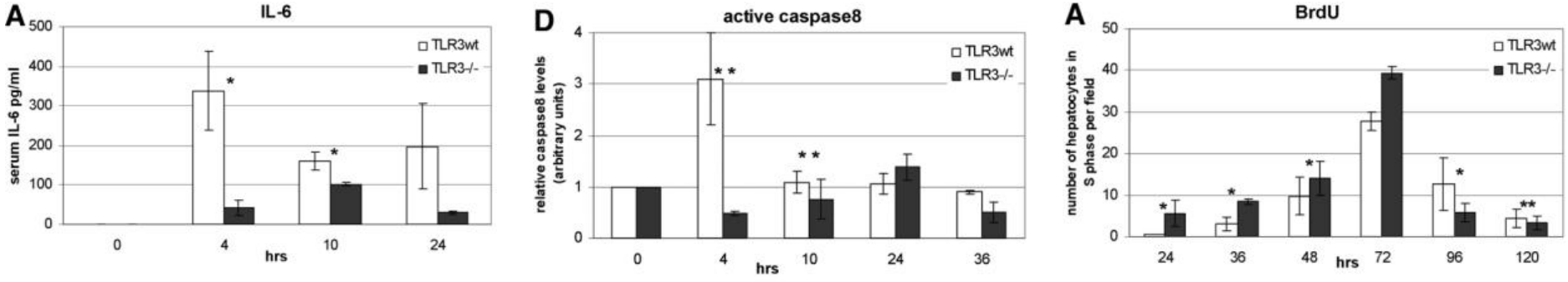
TLR3ko Mice are Protected from Liver Inflammation

- TLR3 deficiency attenuates liver injury induced by ConA in NOD mice



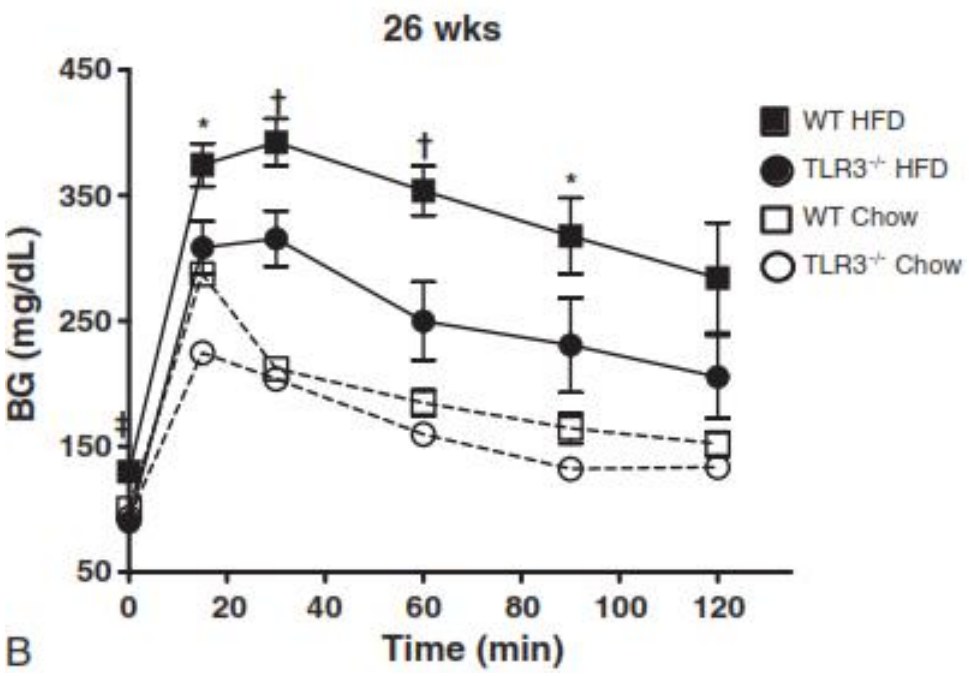
Xiao et al, J Immunol. 2009

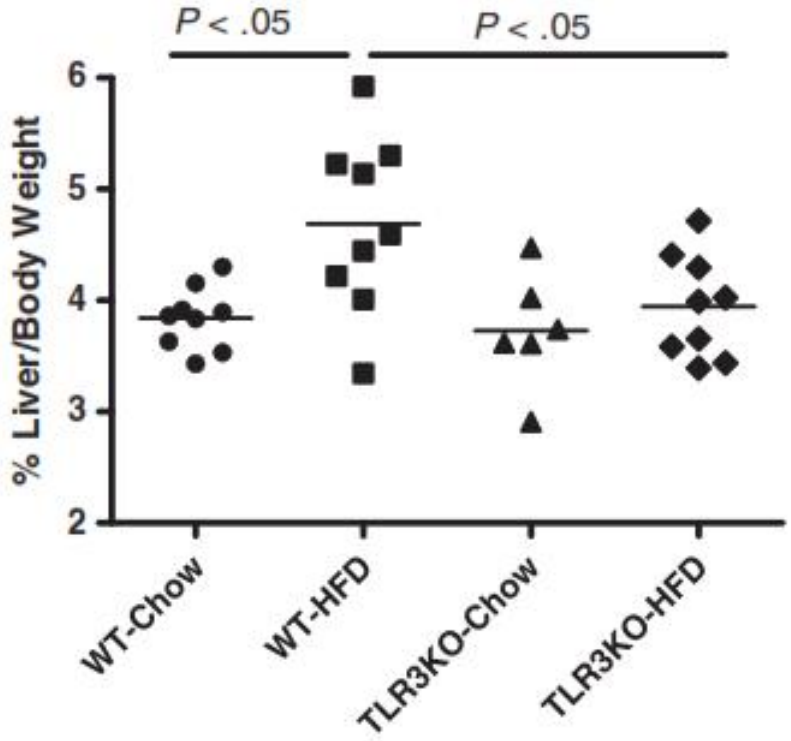
- TLR3 deficiency attenuates inflammation and hepatocyte activation/apoptosis, leading to improved liver regeneration post partial Hepatectomy in mice



Zorde-Khvalesky et al, Hepatology. 2009

TLR3ko Mice are Protected from Development of Diet-induced Insulin Resistance.





Wu et al, Metabolism. 2012

Rational for Blocking TLR3 in Inflammatory Pathologies

- In some inflammatory diseases, human **TLR3 is over-expressed** on epithelial cells of patients, suggesting a chronic stimulation of TLR3

- TLR3ko or pAb TLR3-treated mice are protected in some inflammatory disease models

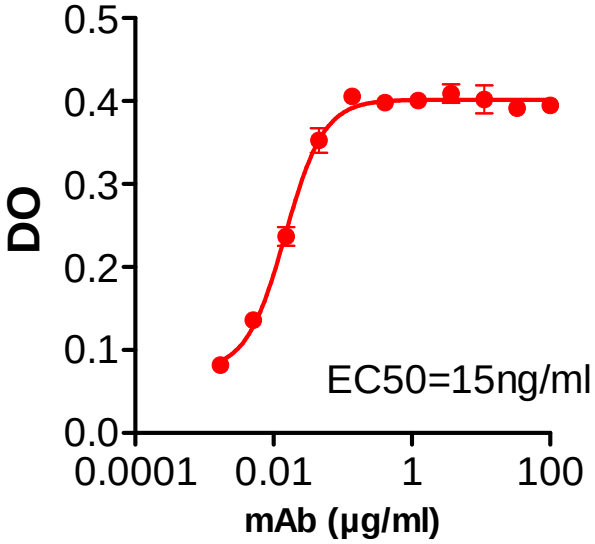
- Some inflammatory pathologies are linked to initial bacterial or **viral infections**, in which TLR3 over-activation can yield to tissue pathological inflammation

- The link with IFN type I, as well as neutrophils infiltration, highlights the potential role of the TLR3 pathway
  - **Necrotic cells**, including neutrophils, are able to induce inflammatory factors, in a TLR3-dependant manner
  - **IFN type I** strongly up-regulated TLR3 expression in immune cells and in epithelial cells

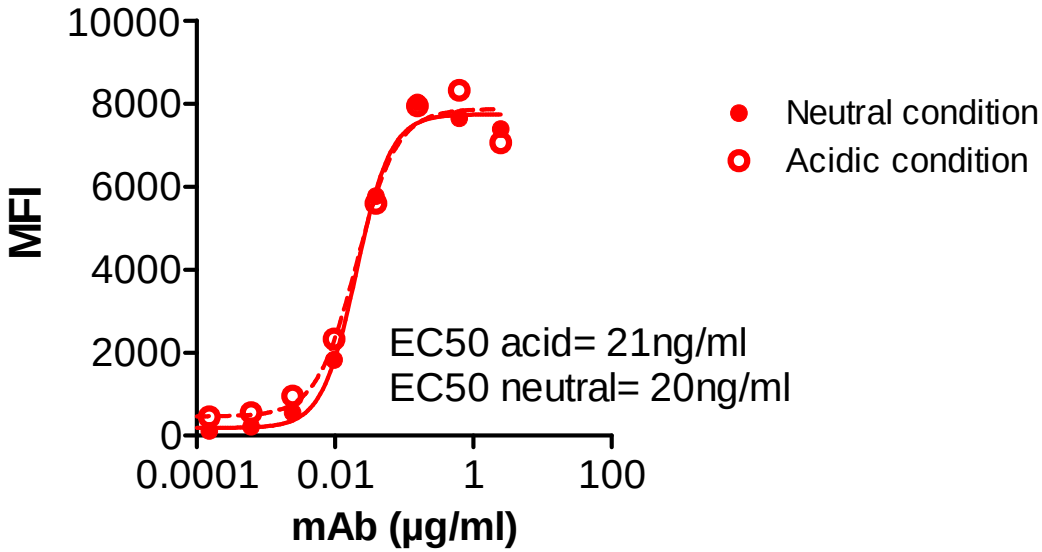
Anti-human TLR3 mAbs Generation: IPH33 Program

- Anti-human TLR3 mAb were generated by classical mouse or rat immunization with recombinant TLR3–ECD protein
- mAbs were screened for their binding to TLR3

ELISA on rec-huTLR3-ECD

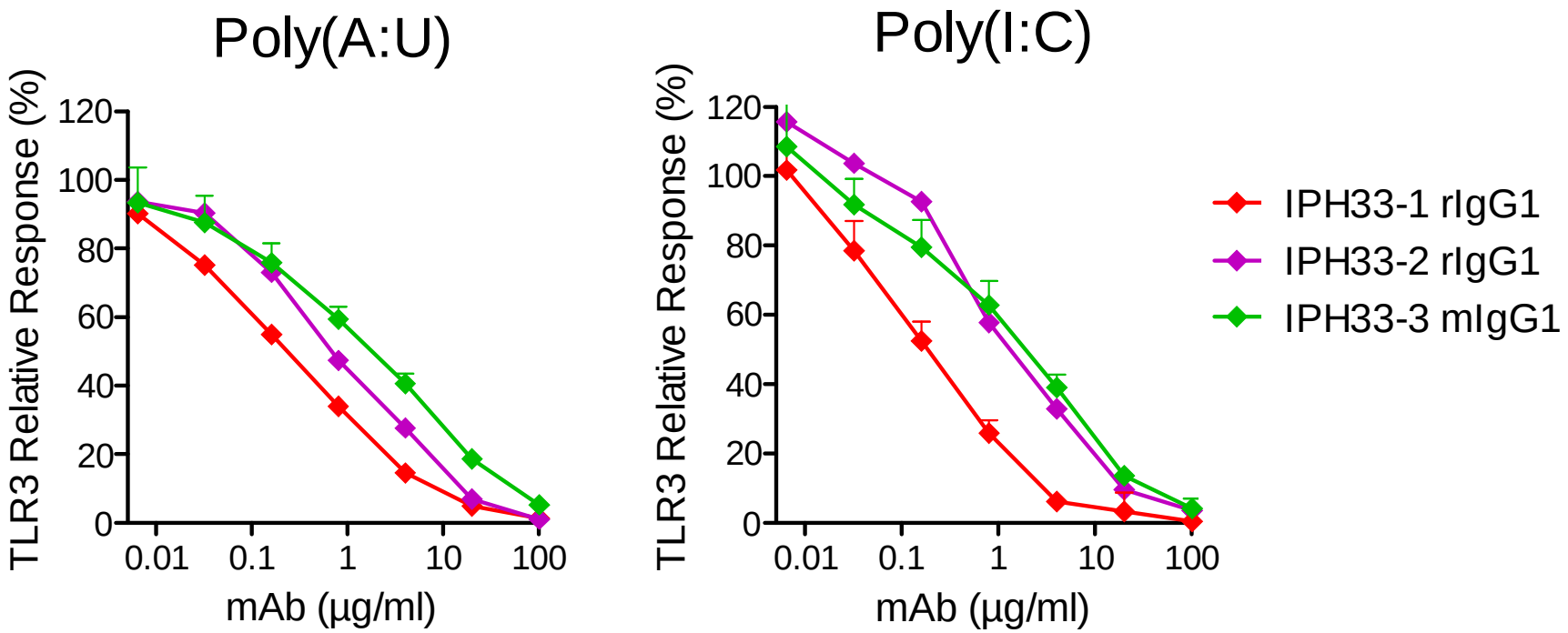


FACS on surface TLR3-CD32



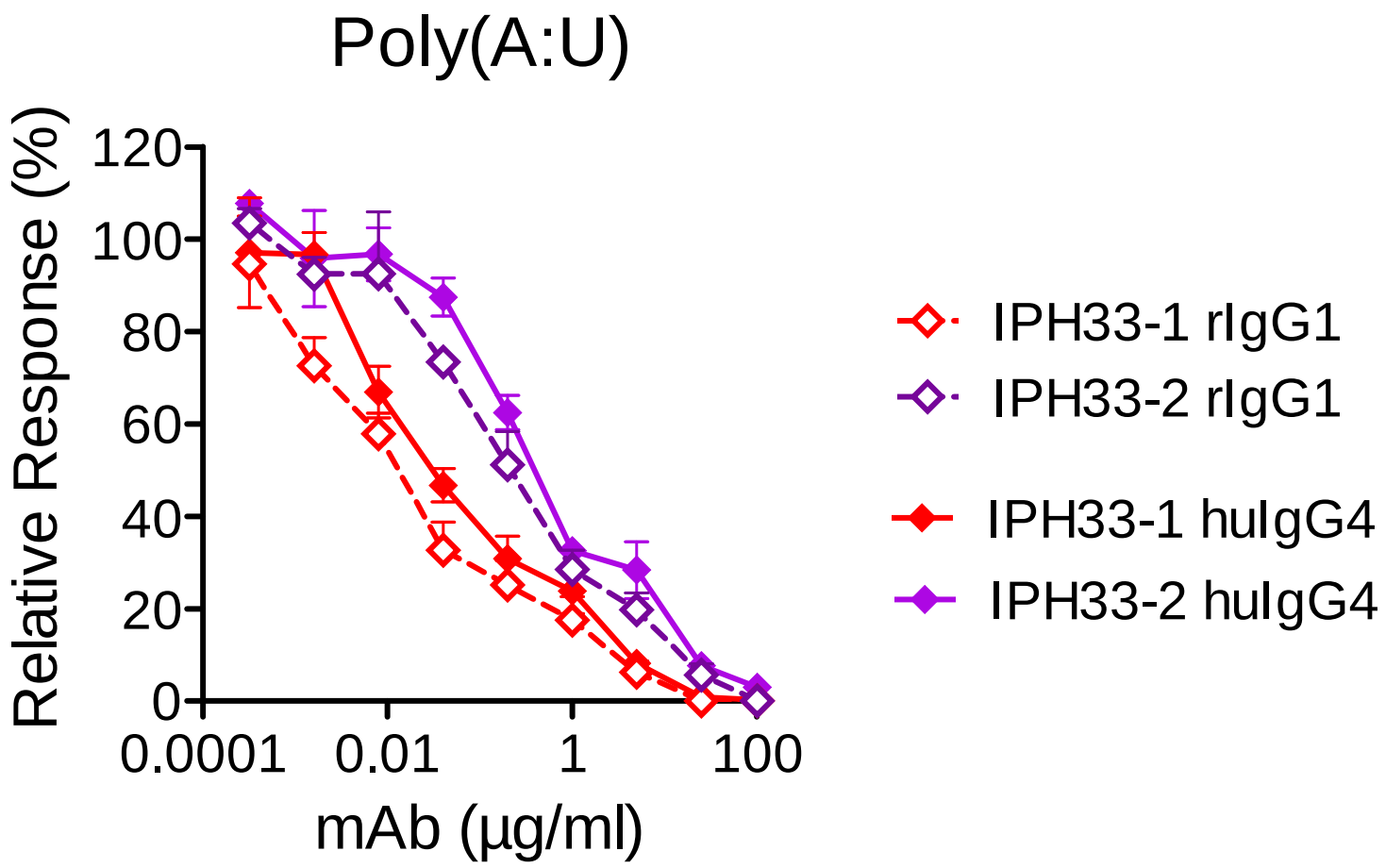
- mAbs of interest were then selected on their ability to block dsRNA signaling in a **human and cynomolgus TLR3** luciferase assay

Anti-TLR3 mAbs Block dsRNA-induced signaling in Human TLR3 Luciferase Assay



- Similar results with cyno TLR3
- No cross-reaction on mouse TLR3
- No cross-reaction on other TLRs

Humanized Anti-TLR3 mAbs (hu-IgG4) Block dsRNA-induced Signaling in Human TLR3 Luciferase Assay

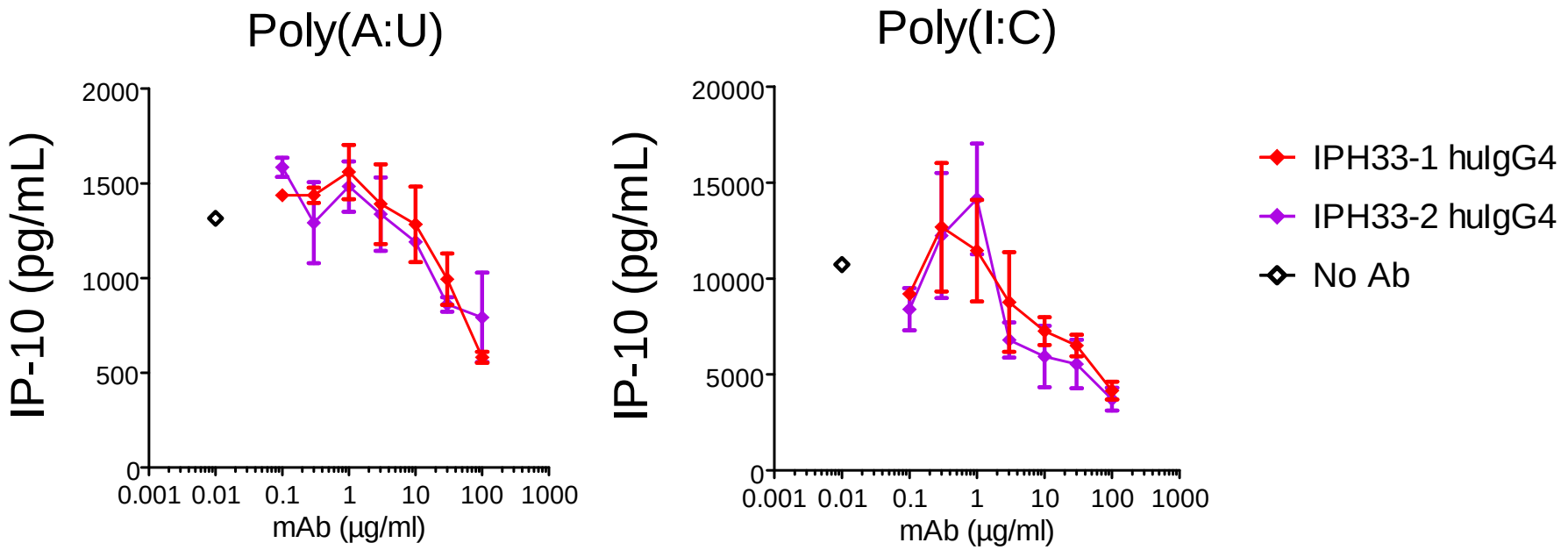


IPH33-1 and IPH33-2 were humanized as human IgG4 with S241P

Humanized Anti-TLR3 mAbs (hu-IgG4) Block dsRNA-induced Signaling in Human PBMC

Healthy Donor PBMC

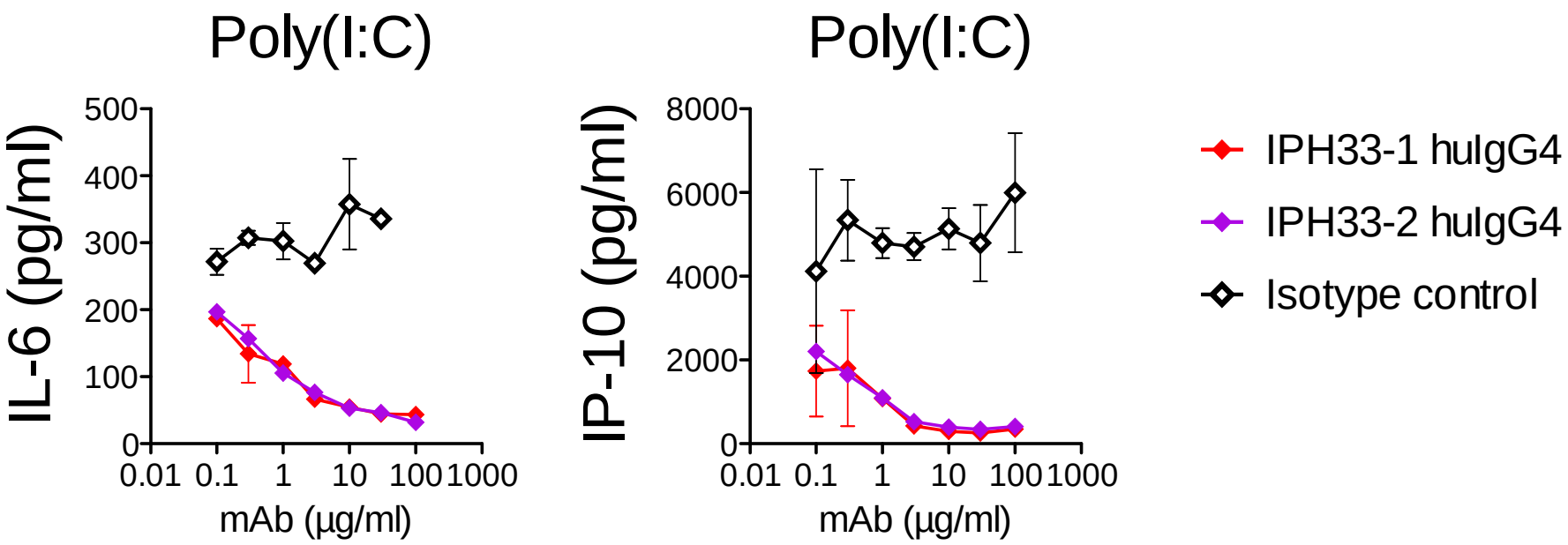
(0.018% +/- 0.015 TLR3+ BDCA3+ DC)



- Poly(I:C) dsRNA activates both TLR3 and RIG-Like Helicase Receptors

Humanized Anti-TLR3 mAbs (hu-IgG4) Block dsRNA-induced Signaling in Human Normal Epithelial Cells

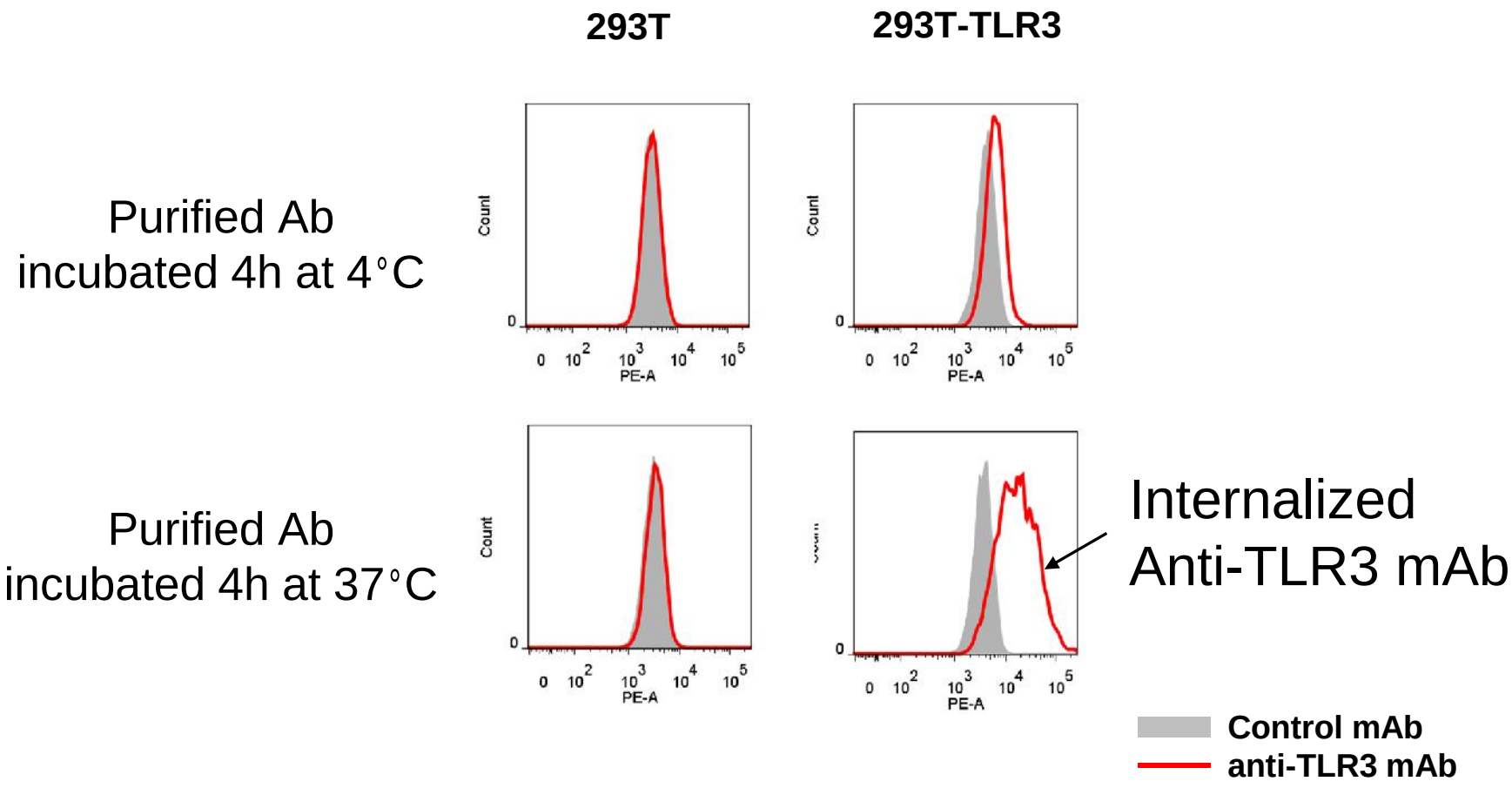
Normal Human Broncho-Epithelial Cells



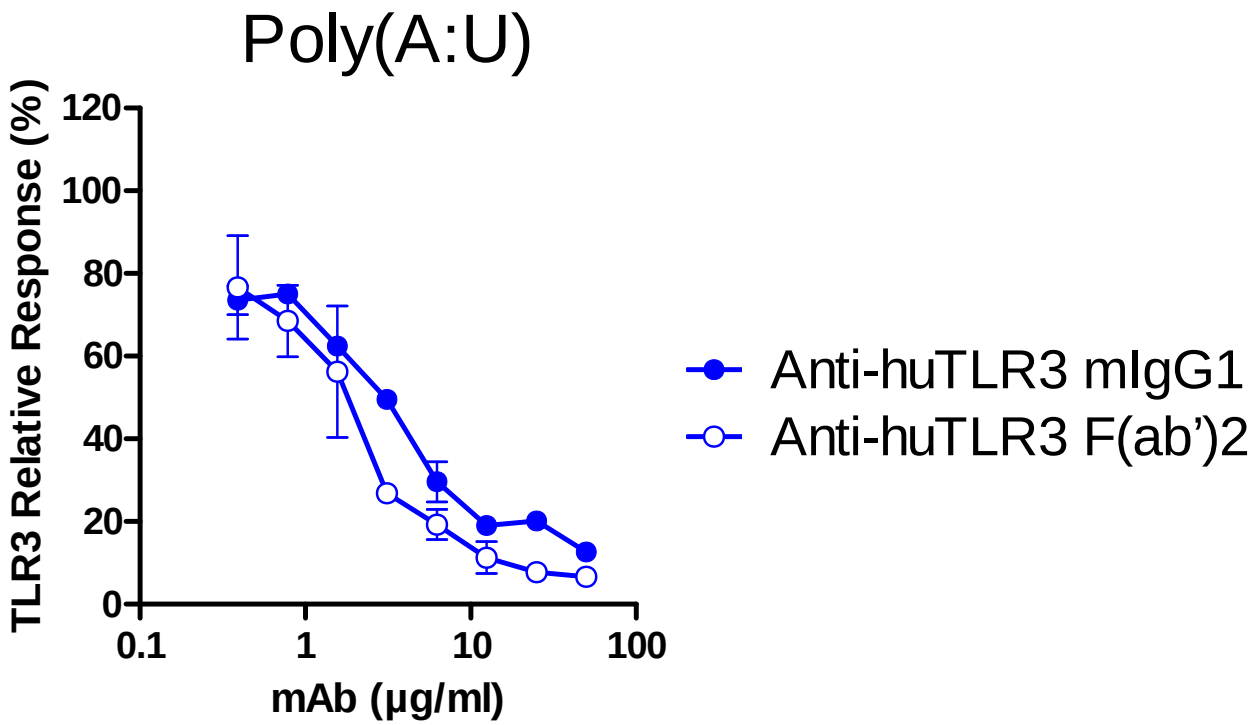
- Poly(I:C) dsRNA activates both TLR3 and RIG-Like Helicase Receptors

Anti-TLR3 mAbs are Specifically Internalized in TLR3-expressing Cells

Intracellular staining

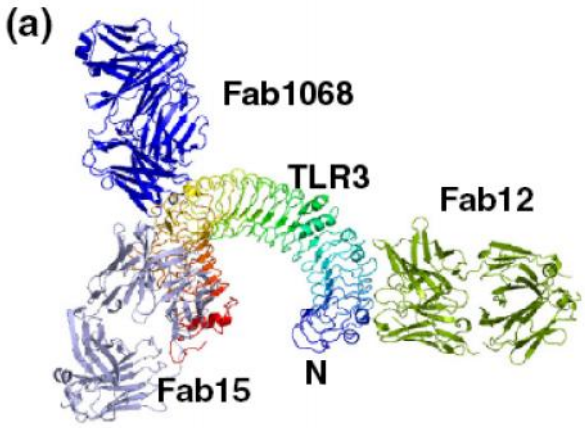


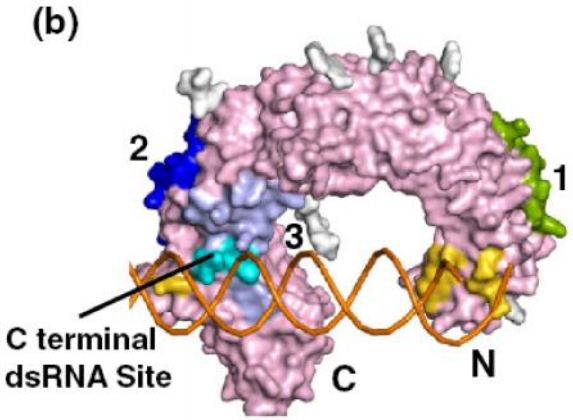
Potency of Blocking Anti-TLR3 mAbs Does Not Depend on Fc-mediated Endocytosis

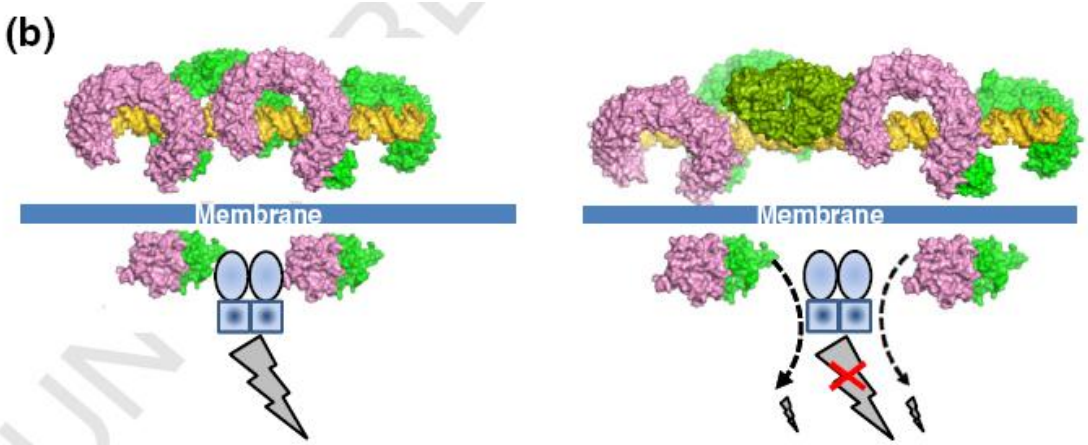


- mIgG1 and F(ab')2 fragments had similar affinity in SPR measurement
- Excess human IgG1 did not impact blocking anti-TLR3 mAbs potency *in vitro* in TLR3 Luciferase Assay

Reported Mode of Action for Anti-TLR3 mAbs





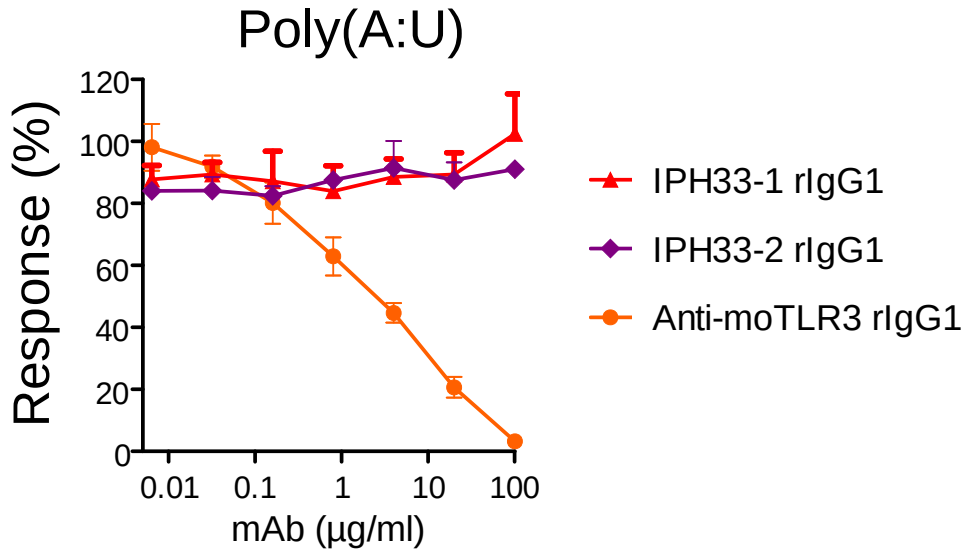


Luo et al, J Mol Biol. 2012

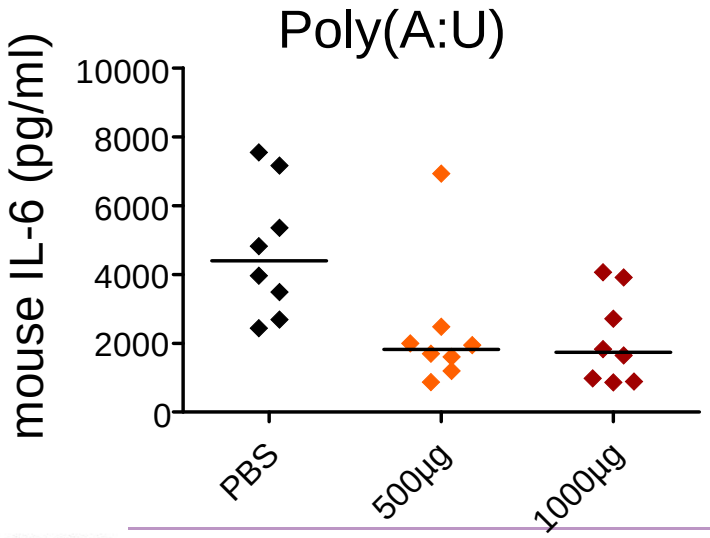
- Fab15 was reported to block binding of dsRNA to recTLR3-ECD, in the C-term dsRNA binding site
- Fab12 was suggested to inhibit TLR3 multimerisation

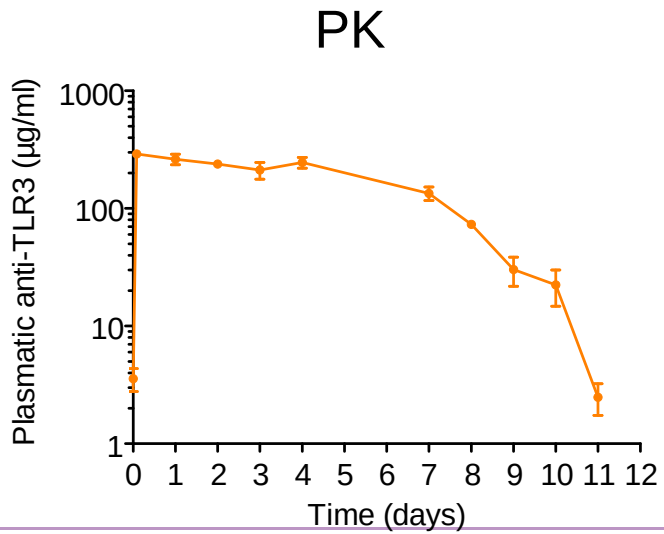
Surrogate Rat Anti-mouse TLR3 mAbs Blocks dsRNA-induced Mouse TLR3 Signaling *in vitro* and *in vivo*

In vitro



In vivo





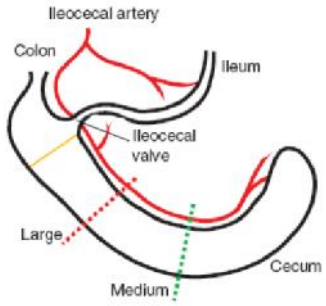
Anti-mouse TLR3 mAb Protects Mice From Septic Shock in Therapeutic Setting

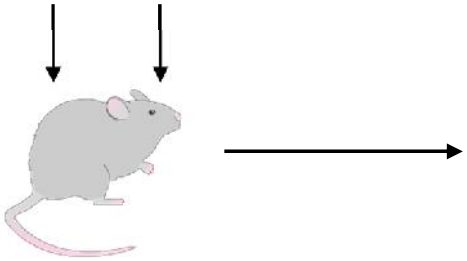
Anti-moTLR3 treatment
at +6h, +24h post CLP

CLP
Medium grade

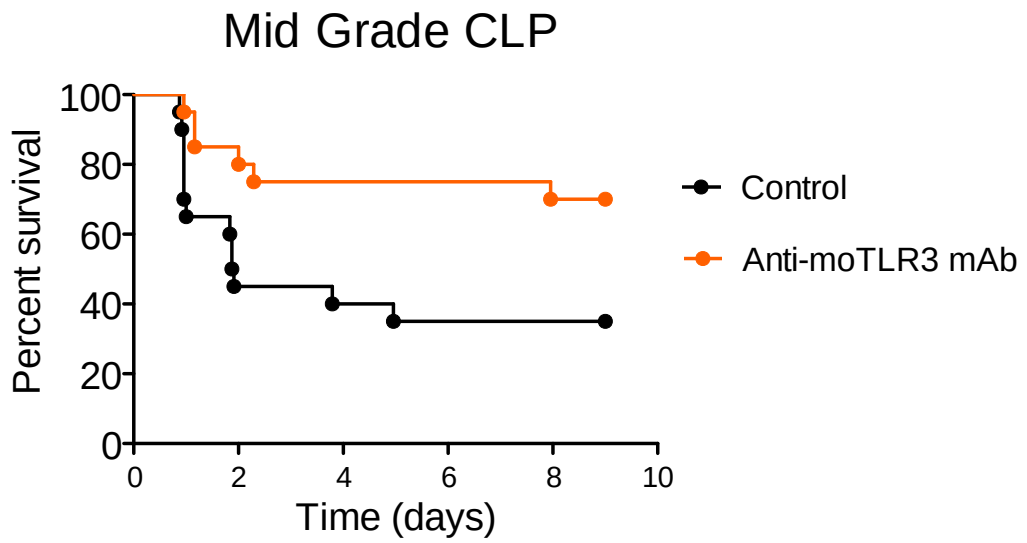
Day 0







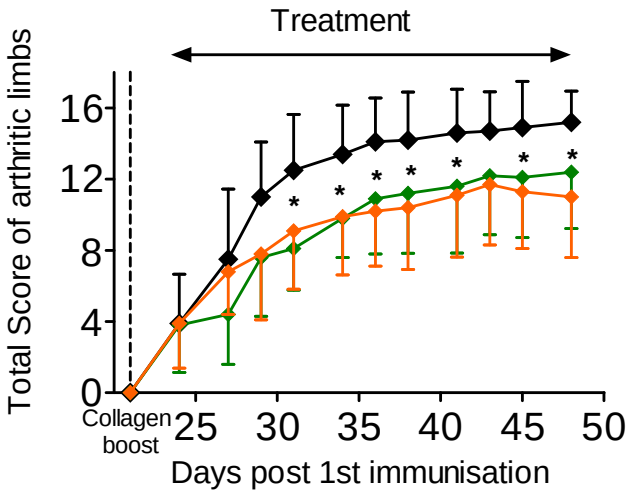
Monitoring
mice survival



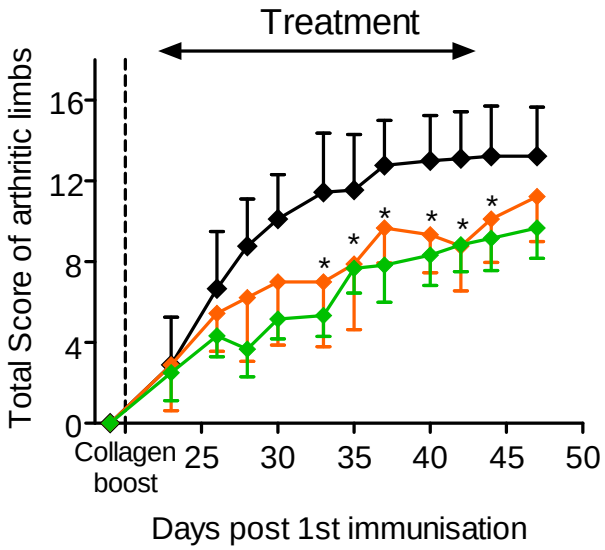
Gehan-Breslow-Wilcoxon Test: * , p= 0.013

Anti-mouse TLR3 mAb Reduces Arthritis in Collagen-Induced Mouse Model in Therapeutic Setting





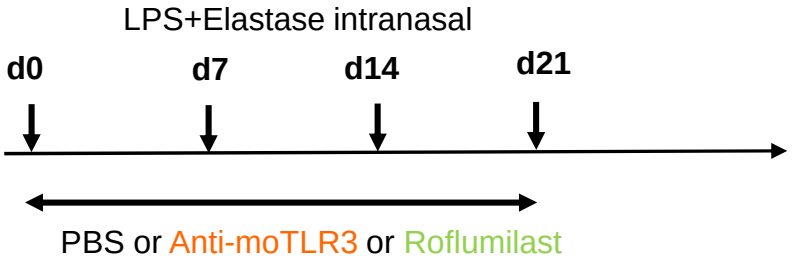
- Control
- Anti-mouse TLR3 mAb
- Methotrexate



- Control mAb
- Anti-mouse TLR3 mAb
- Anti-TNF mAb (Humira^{TM})

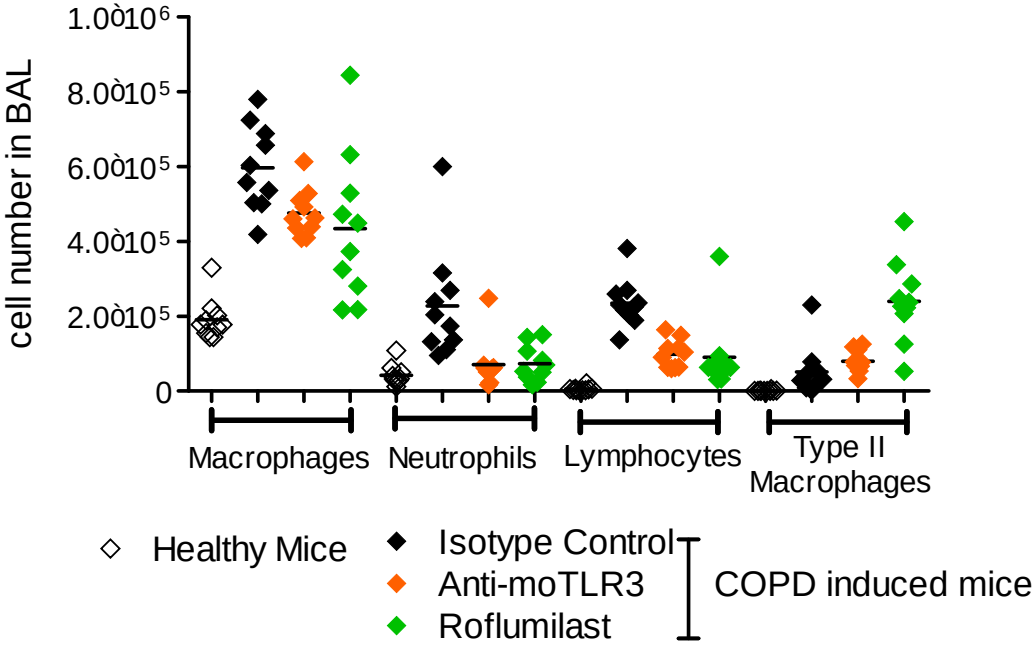
Anti-mouse TLR3 mAb reduces COPD in a LPS-Elastase-induced Model in a Preventive Setting



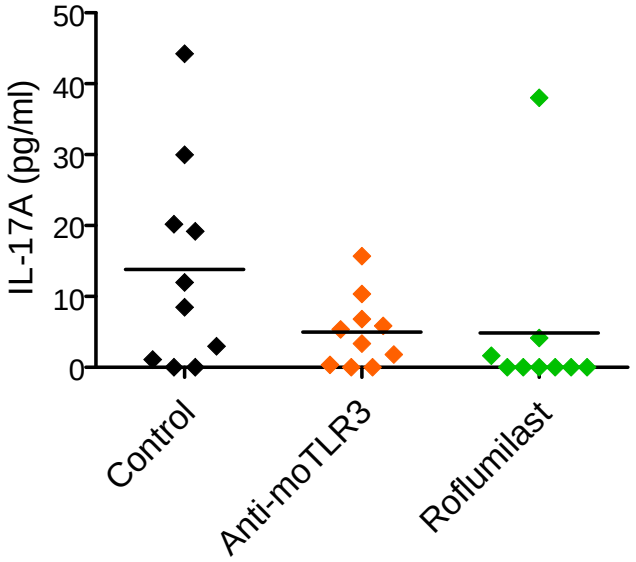


Day 28: Cell infiltration in BALF
Cytokine dosage in BALF
Histopathology on lung

Cell infiltration in BAL



IL-17 in BAL



Lung Histology in LPS-Elastase induced COPD Model

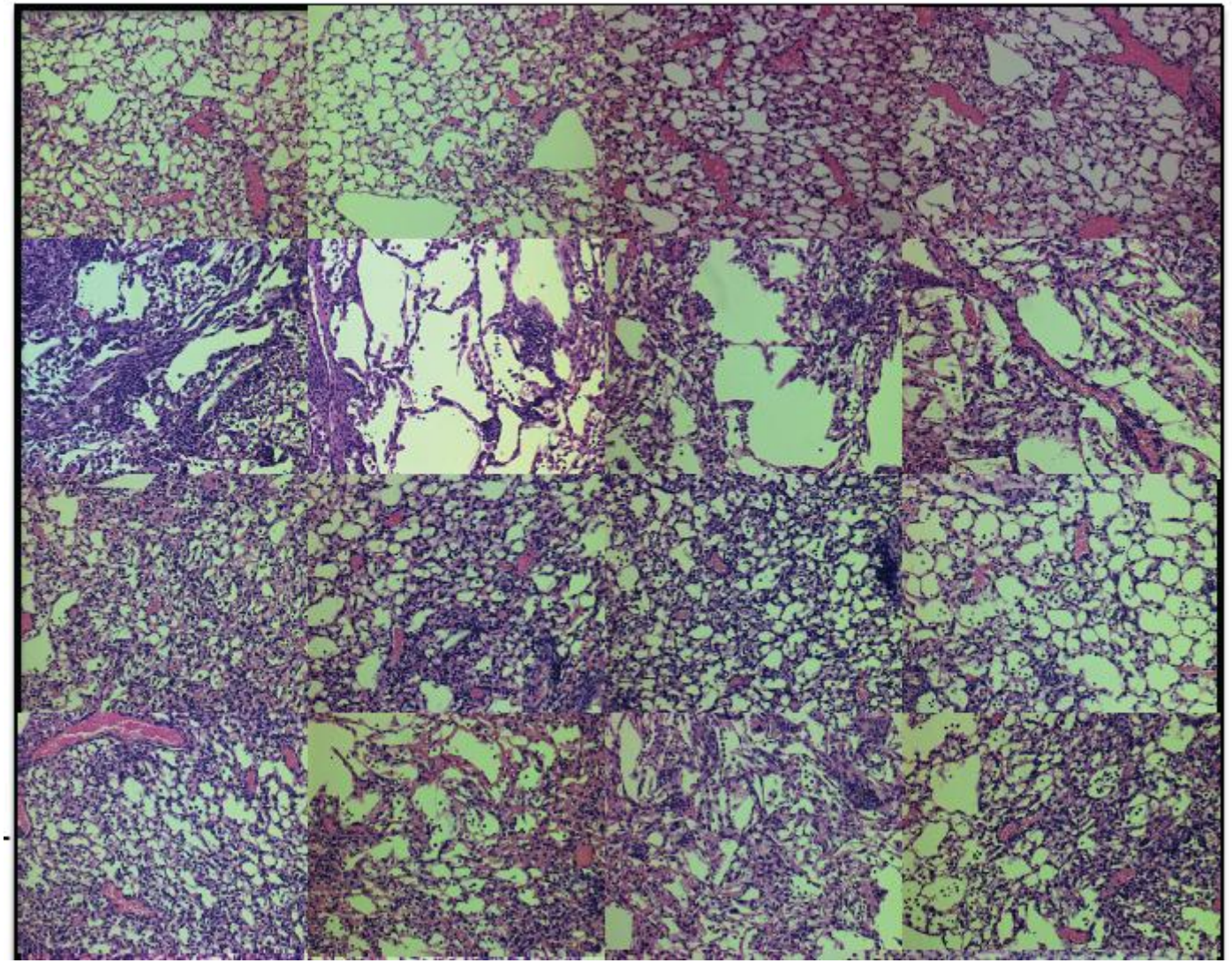
Healthy mice

COPD-induced mice

Control

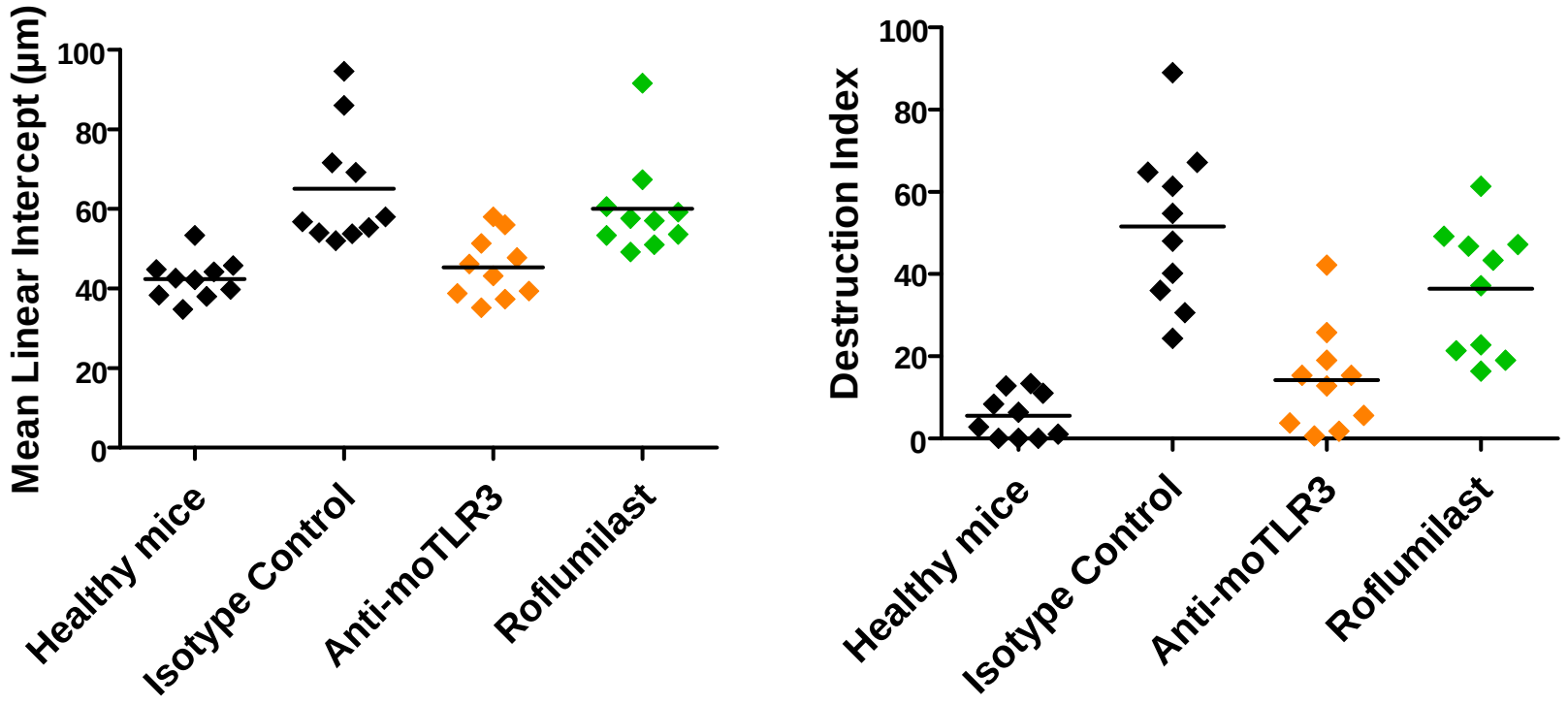
Anti-moTLR3

Roflumilast



Anti-mouse TLR3 mAb reduces COPD in a LPS-Elastase-induced Model in a Preventive Setting

Lung destruction



The Mean Linear Intercept (MLI) : number of alveolar intersects along a grid line divided by the length of each line

Destruction index : Tissue structure is classified as Normal or Destroyed at each intersecting point of the grid (n=108) . DI=D/(D+N)*100.

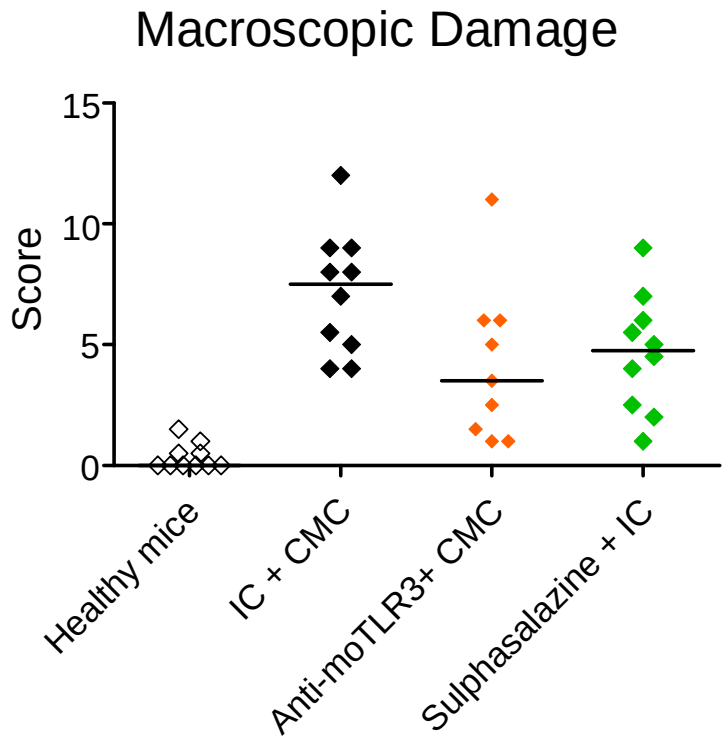
Anti-mouse TLR3 mAb attenuates Colitis in a DSS-induced Model

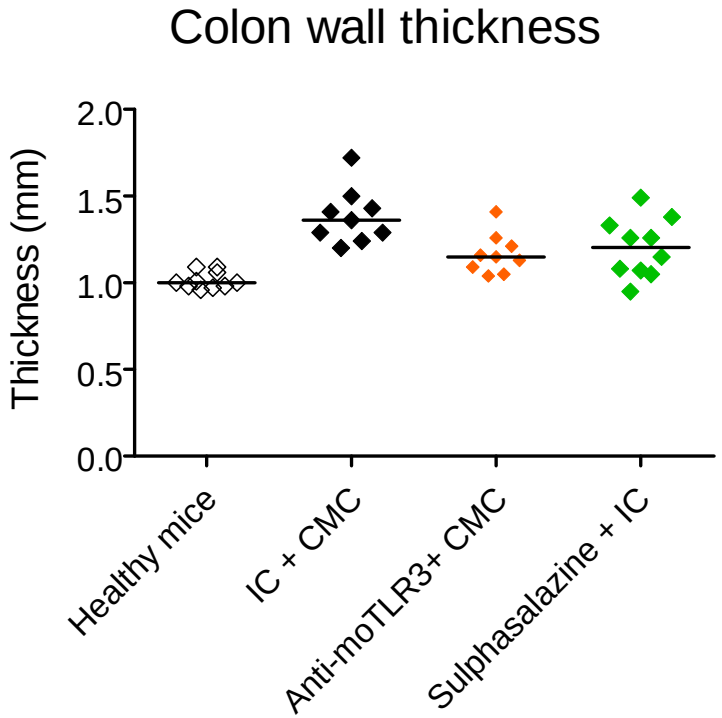


DSS 5% in drinking water

Day 6 : Sacrifice and scoring

PBS or Anti-moTLR3 or Sulfazalazine





No effect of anti-moTLR3 Ab treatment on weight loss

Anti-mouse TLR3 mAb attenuates Colitis in a TNBS-induced Model in combination with Dexamethasone

Mild Intracolic TNBS



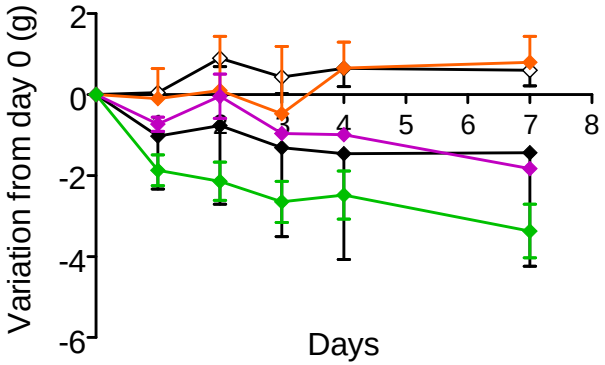
d0

Blind scoring for disease activity, weight loss and survival

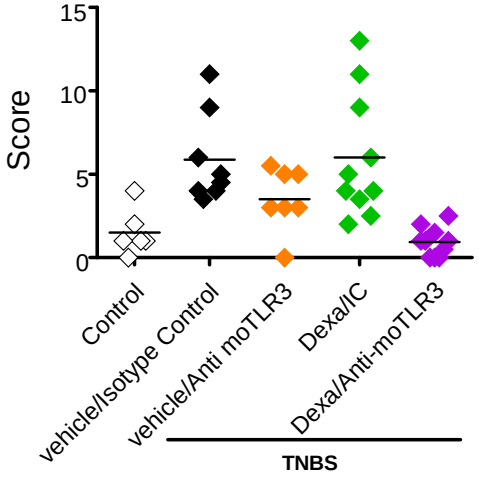
Day 7: tissue collected

Control mAb or Anti-moTLR3 or Dexamethasone or Combination

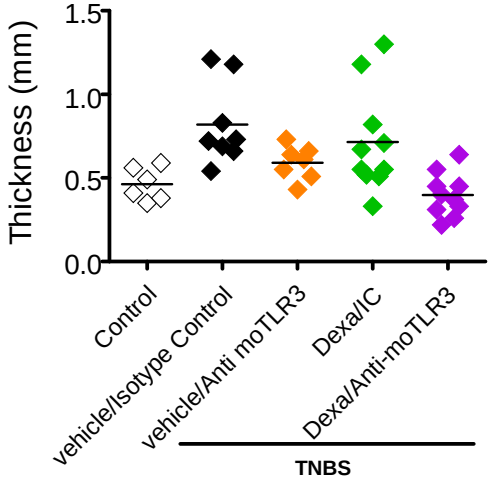
Body weight



Macroscopic damage



Colon wall thickness



Summary

- TLR3 is a receptor for dsRNA that is over-expressed in some inflammatory diseases suggesting a chronic stimulation
- Various models of inflammation in TLR3ko mice support the development of a blocking mAb against TLR3
- POC for therapeutic TLR3 blocking mAb in inflammation models of RA, COPD and Colitis has been established with anti-mouse TLR3 surrogate mAb
- One anti-TLR3 (CNTO-3157) is currently being tested in Phase I/II in Healthy Normal and Asthmatic Participants Inoculated With Human Rhinovirus Type 16 at 10-3mg/kg
- A humanized anti-TLR3 mAb (IPH33-1) blocking TLR3 signaling with potent efficacy has been generated
- Next steps for IPH33-1
    - Preliminary PK/PD in NHP
    - IND-enabling regulatory studies