GLIADIN BINDING TO CXCR3 INDUCES A MyD88- AND G PROTEIN SIGNALING-DEPENDENT INCREASED INTESTINAL PERMEABILITY AND ZONULIN RELEASE

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Background & premises

- Celiac disease (CD) is an auto-immune enteropathy triggered by the ingestion of gluten. **Gliadin** is the toxic component of the grain protein gluten

- In susceptible individuals, the interplay between gliadin and intestinal epithelial cells triggers tight junction disassembly and as a result **increased intestinal permeability**, which is considered an early crucial biological event in the pathogenesis of CD

- **Zonulin** induces tight junction disassembly. It is therefore considered to be involved in CD

- In **CD**: An increased and persistent release of zonulin and a significant increase in intestinal permeability occur (S. Drago et al., Scand J Gastroenterol 2006)
  Apical, but not basolateral, exposure to gliadin leads to zonulin release (MG Clemente et al., Gut 2003)

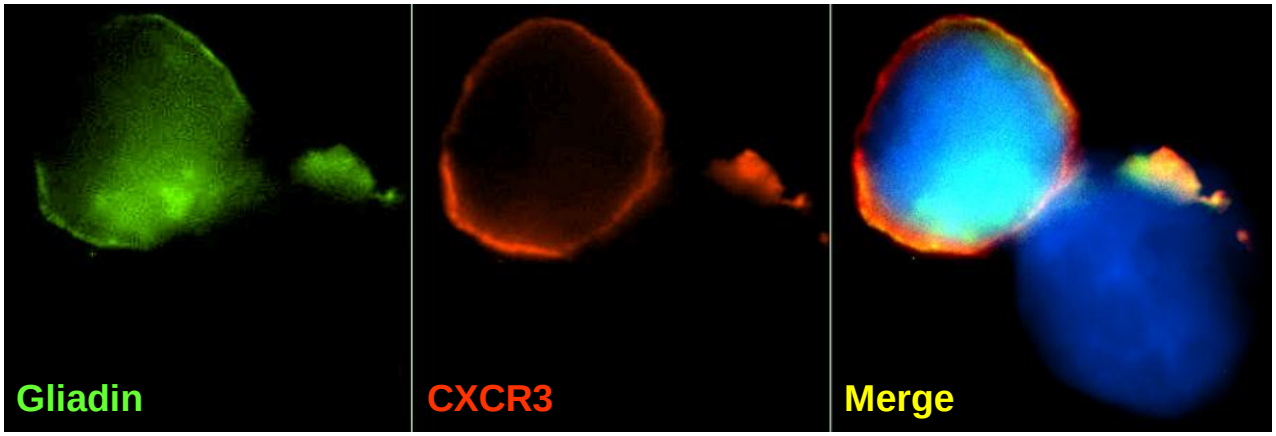
- We recently identified the chemokine receptor **CXCR3** as the receptor to which gliadin binds

Aim

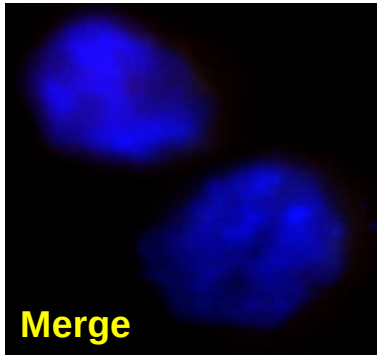
To explore the function of CXCR3 after gliadin binding

Co-localization of PT-gliadin and CXCR3

CXCR3-transfected HEK cells
PT-gliadin treatment

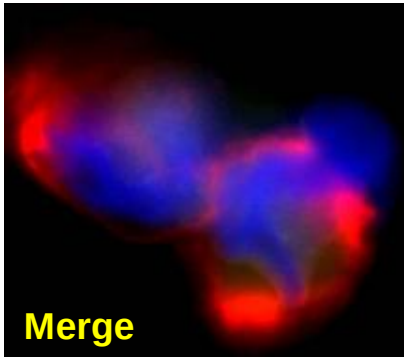


pcDNA-transfected cells
PT-gliadin treatment



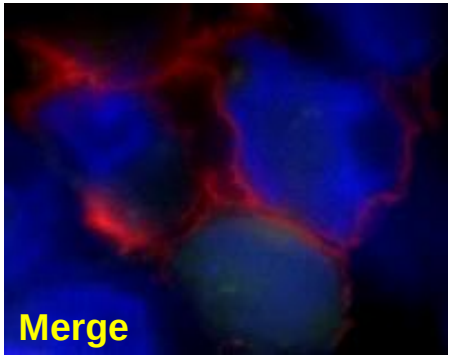
Gliadin
CXCR3

CXCR3-transfected cells
PT-gliadin treatment



Anti-FITC
CXCR3

CXCR3-transfected cells
BSA treatment

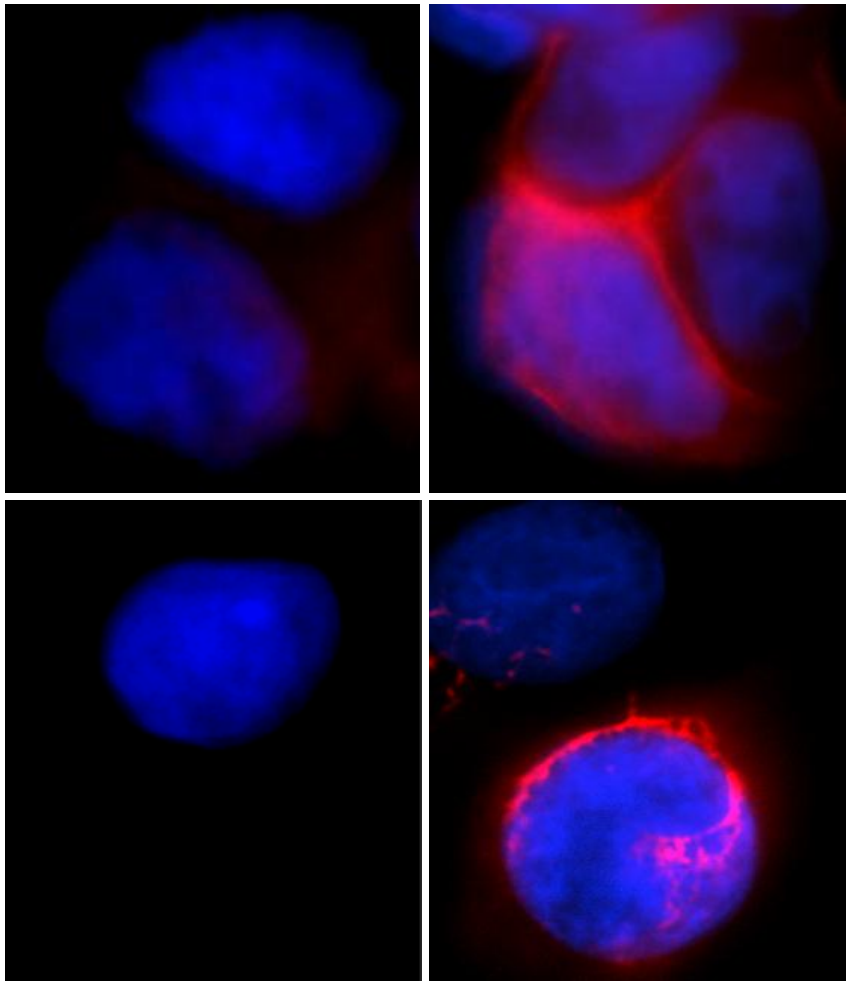


BSA
CXCR3

Basal CXCR3 expression on intestinal epithelial cell lines

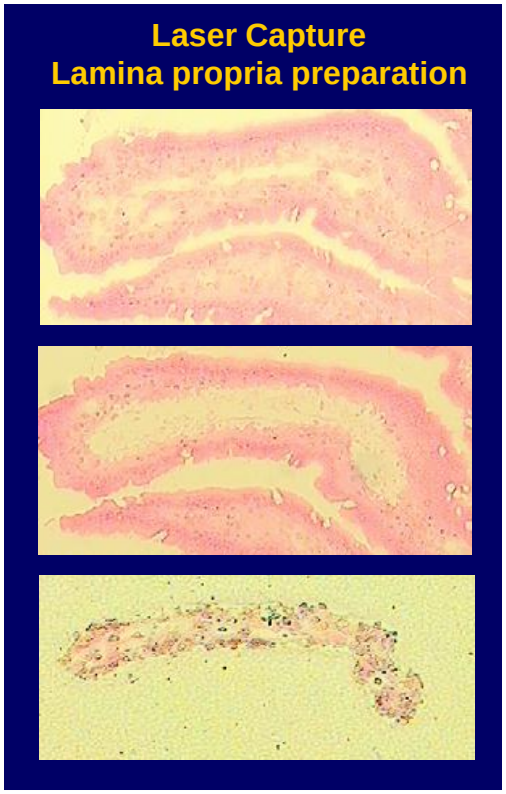
Caco-2

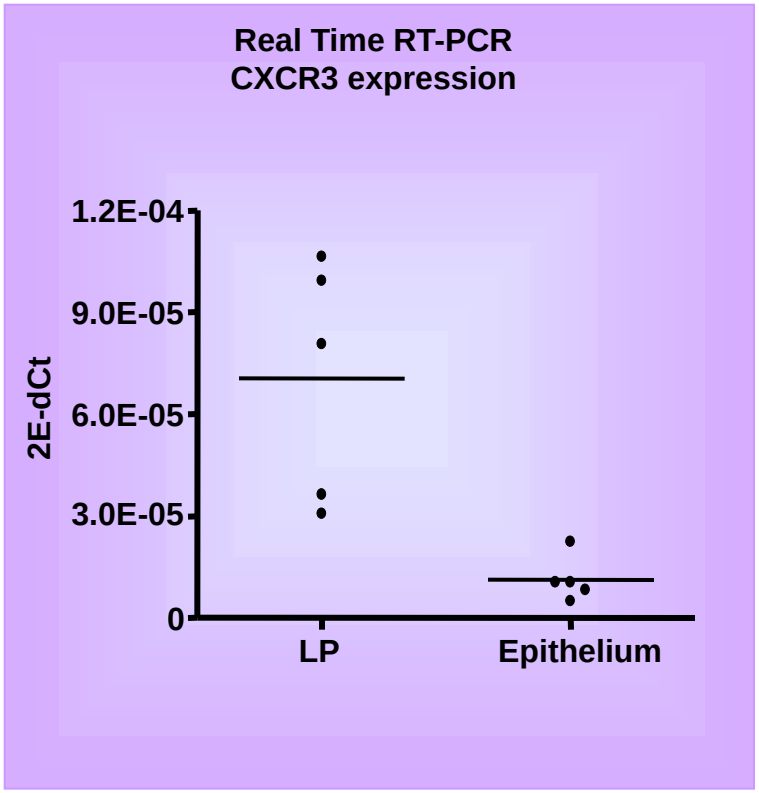
IEC6

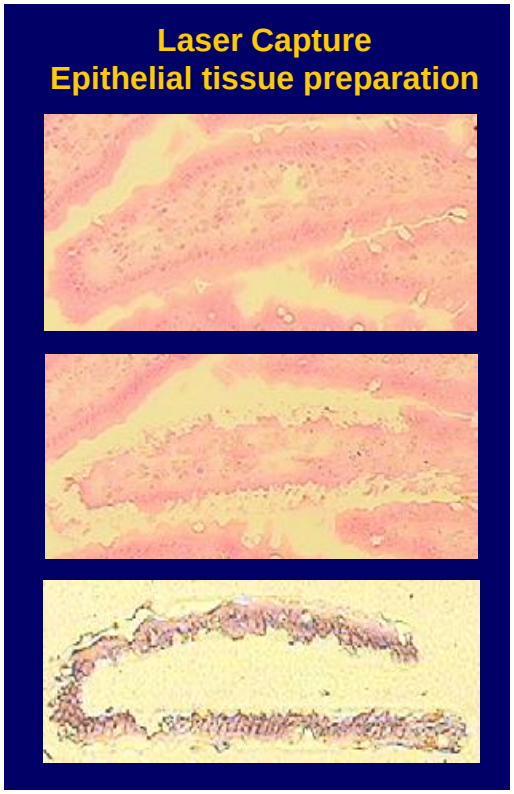


Isotype control CXCR3

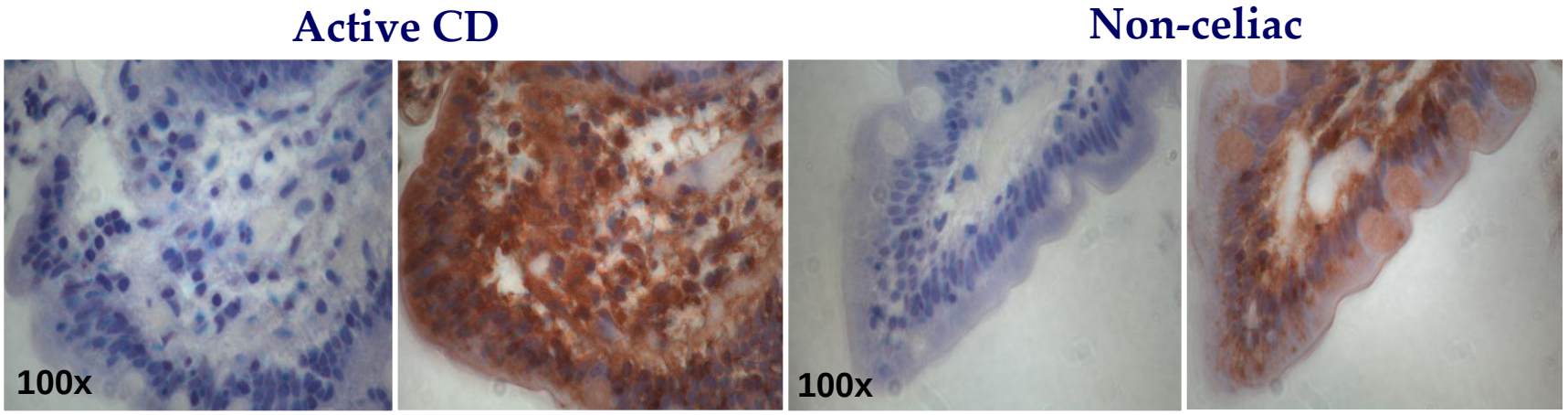
CXCR3 is expressed in mouse intestinal epithelium and in lamina propria

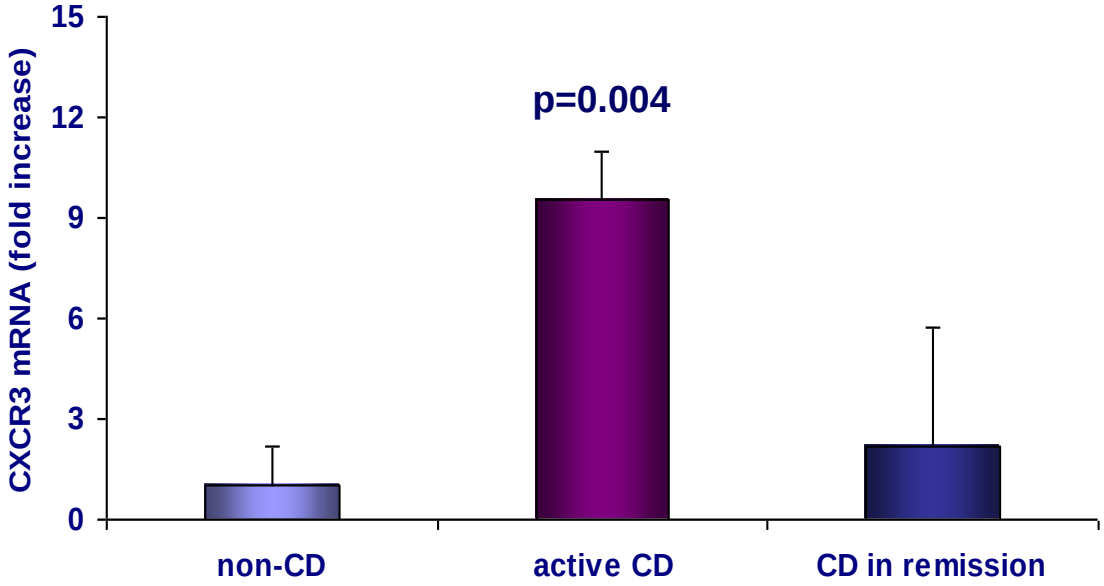




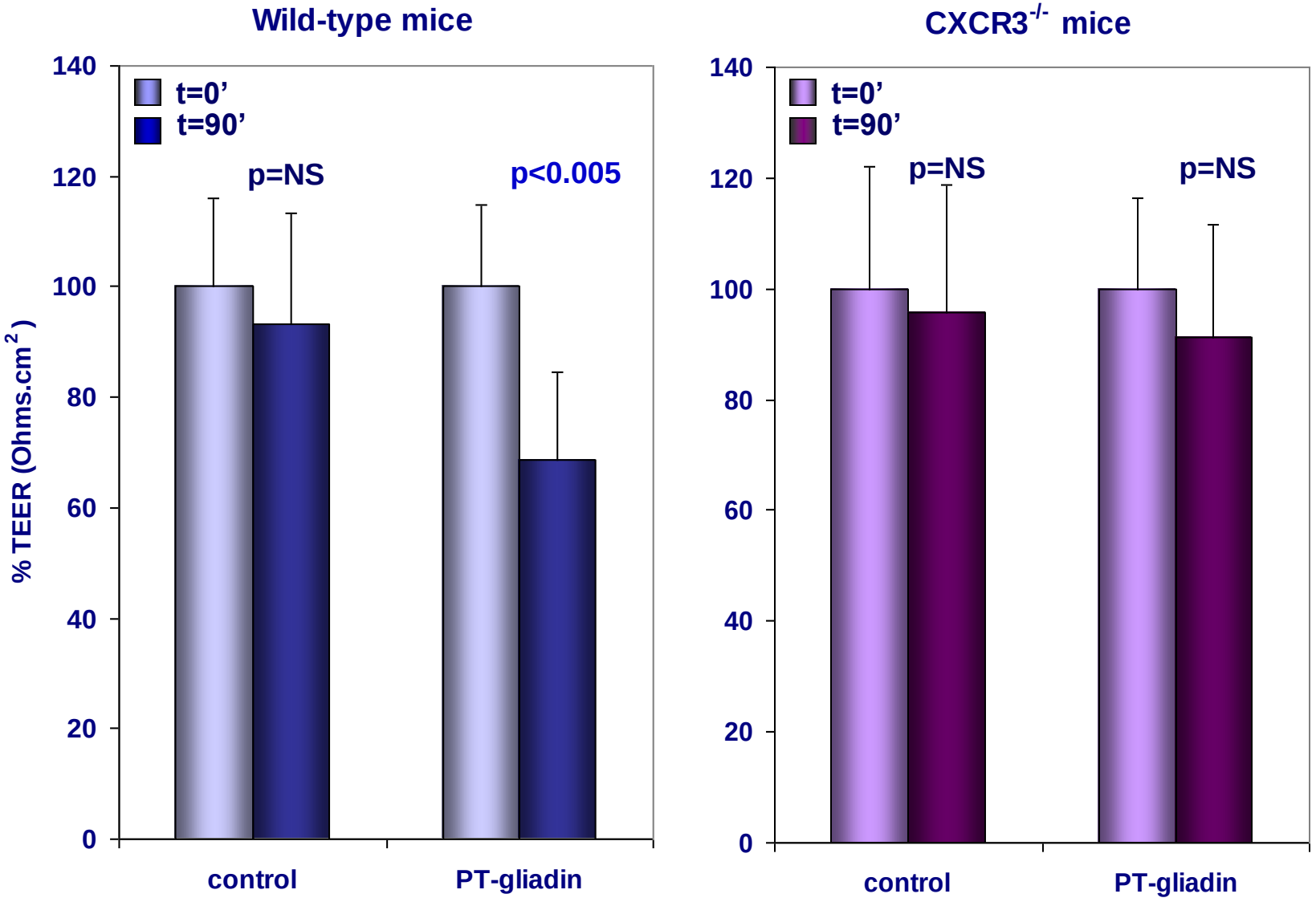


CXCR3 expression in human intestinal tissues

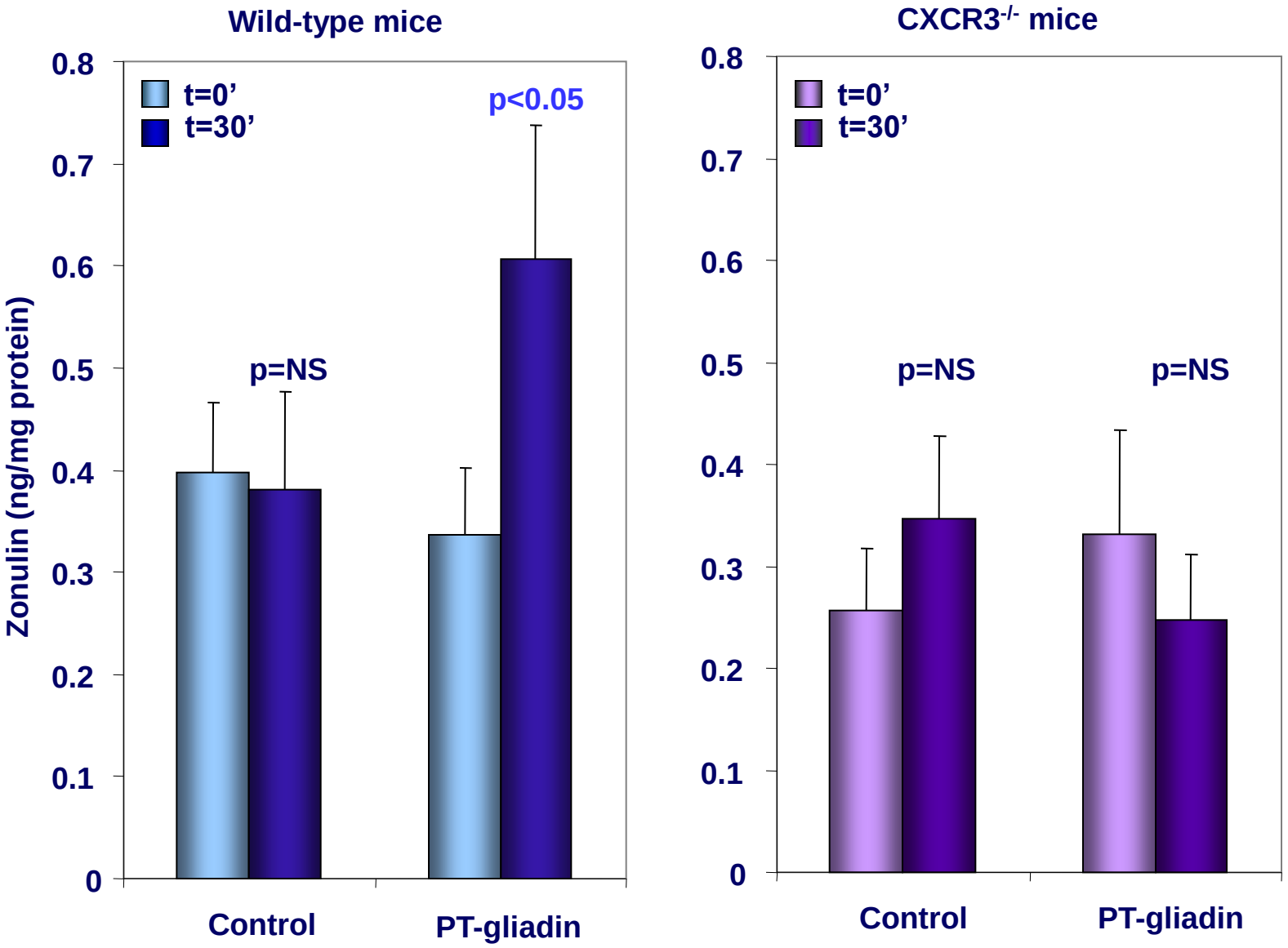




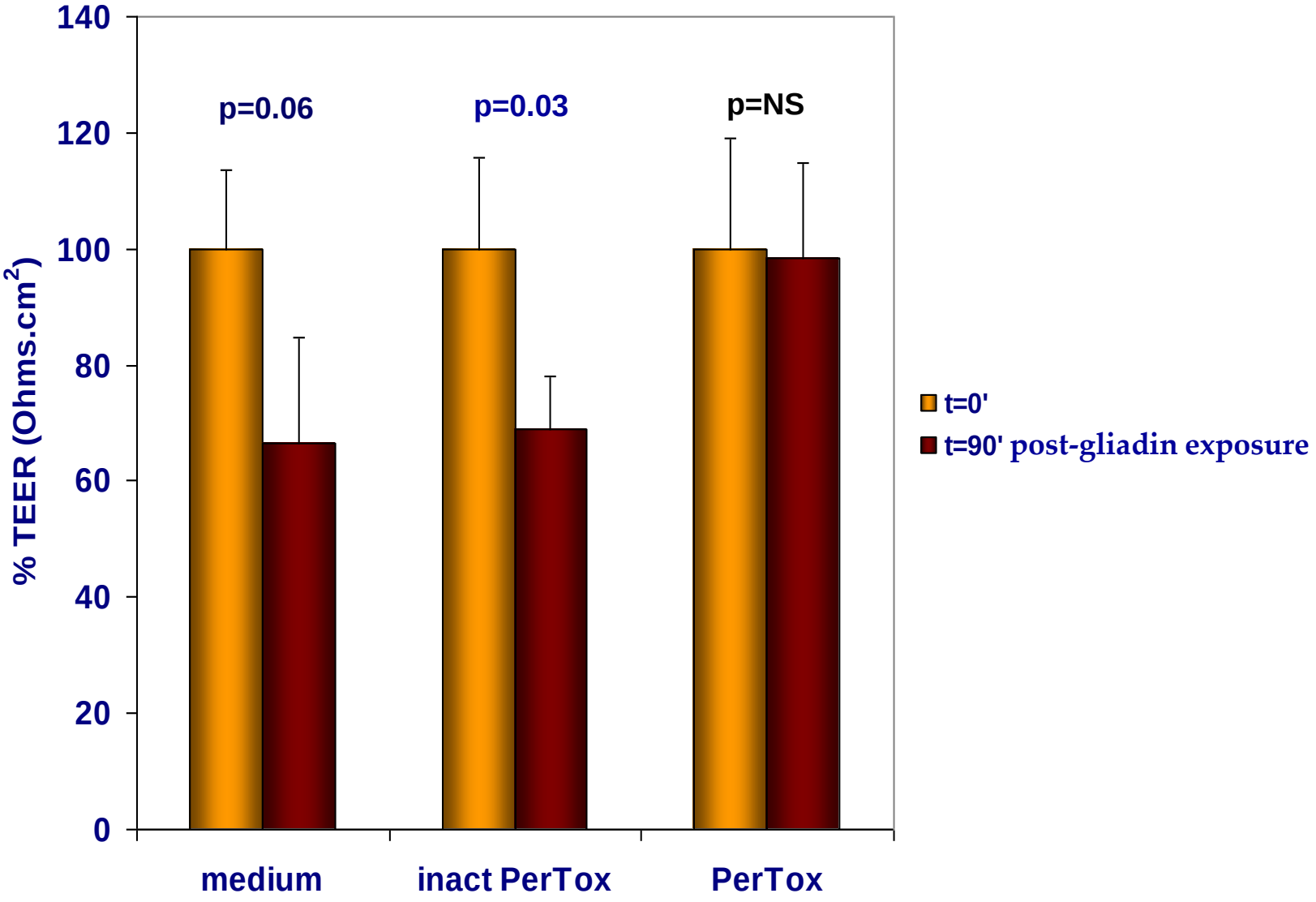
PT-gliadin induces increased permeability in wild-type-, but not in CXCR3$^{-/-}$, intestinal segments



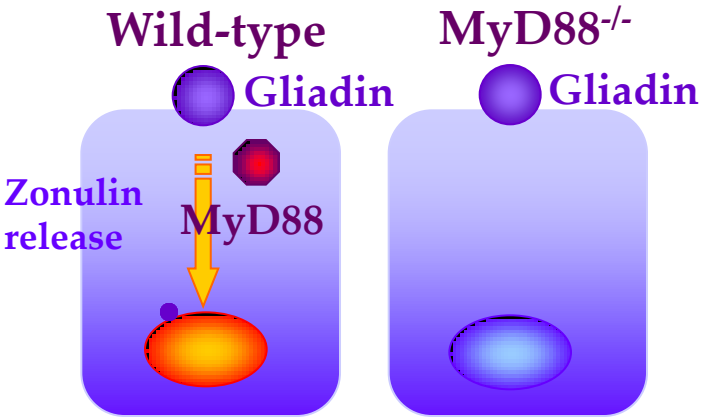
PT-gliadin induces increased zonulin release in wild-type-, but not in CXCR3$^{-/-}$, intestinal segments



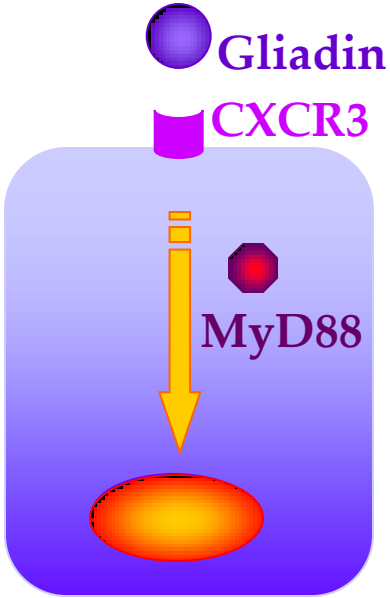
Pre-incubation with *Pertussis toxin* prevents PT-gliadin induced increase of intestinal permeability

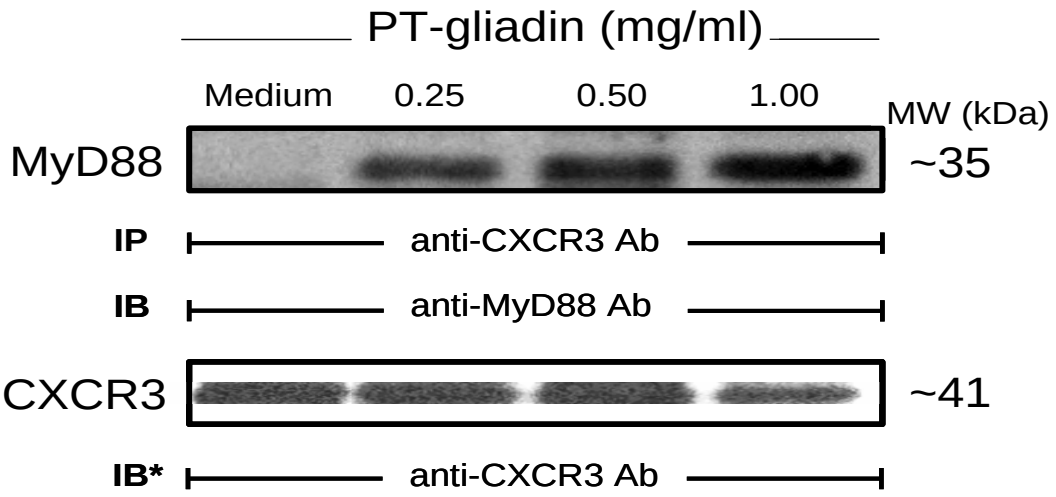


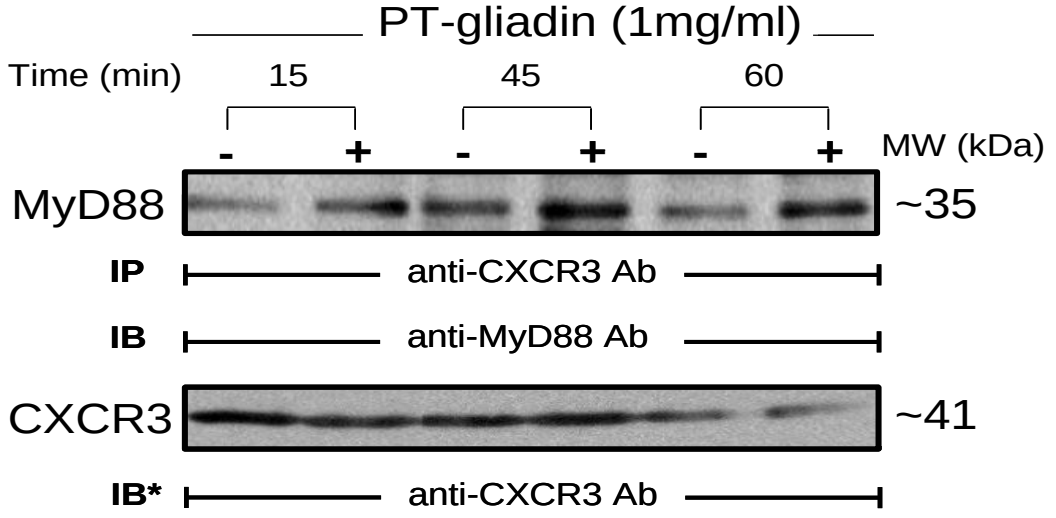
Physical association of CXCR3 and MyD88 after PT-gliadin Binding Dose- and Time-dependent



K Thomas et al., J Immunology 2006







Conclusions

- We have shown **colocalization of CXCR3 and gliadin**
- CXCR3 was expressed at **intestinal epithelial level**
- CXCR3 gene expression in biopsy specimens was **enhanced in active CD** compared to controls, and this expression seemed to return to base-line with GFD

- In functional studies, binding of gliadin to CXCR3 induced **enhanced zonulin release** and **increased intestinal permeability**, an early event in CD pathogenesis

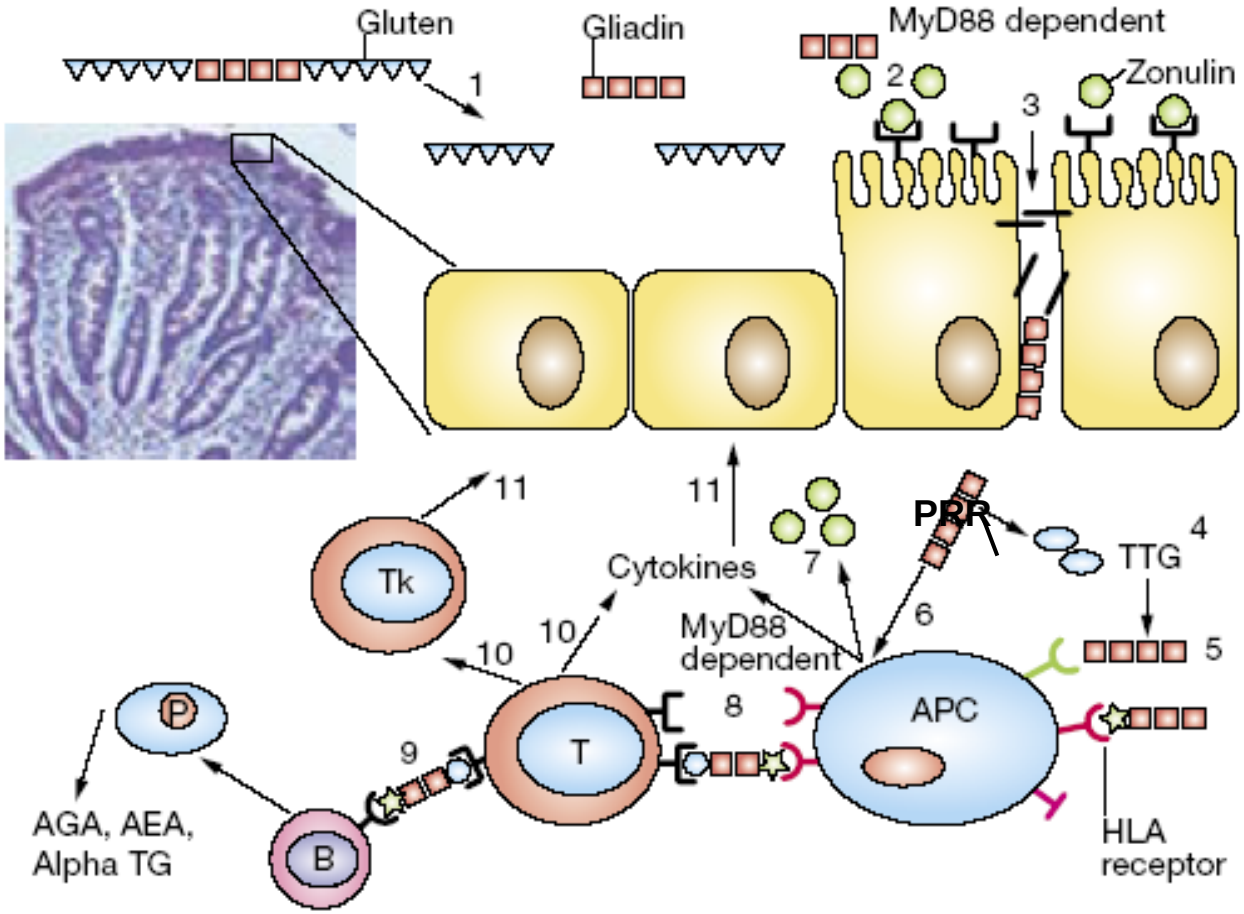
These effects;
- Required **signaling via G protein**

Pre-incubation with G protein signaling-inhibitor pertussis toxin, prevented drop in TEER

- Were **MyD88-mediated**

After gliadin binding, MyD88 is recruited and forms a physical association with CXCR3

Hypothesis



- In genetically predisposed individuals binding of gliadin to CXCR3 is the first step leading to tight junction disruption and subsequent increase in intestinal permeability.

- This allows the passage of gliadin into the intestinal mucosa leading to a cascade of immune events that eventually lead to autoimmunity.

A. Fasano and T. Shea Donohue, Nature Clin Pract Gastroenterol Hepatol 2005