

Role of CXCR3 in Gliadin-mediated Zonulin Release and Increased Intestinal Permeability

¹Mucosal Biology Research Center, University of Maryland School of Medicine,

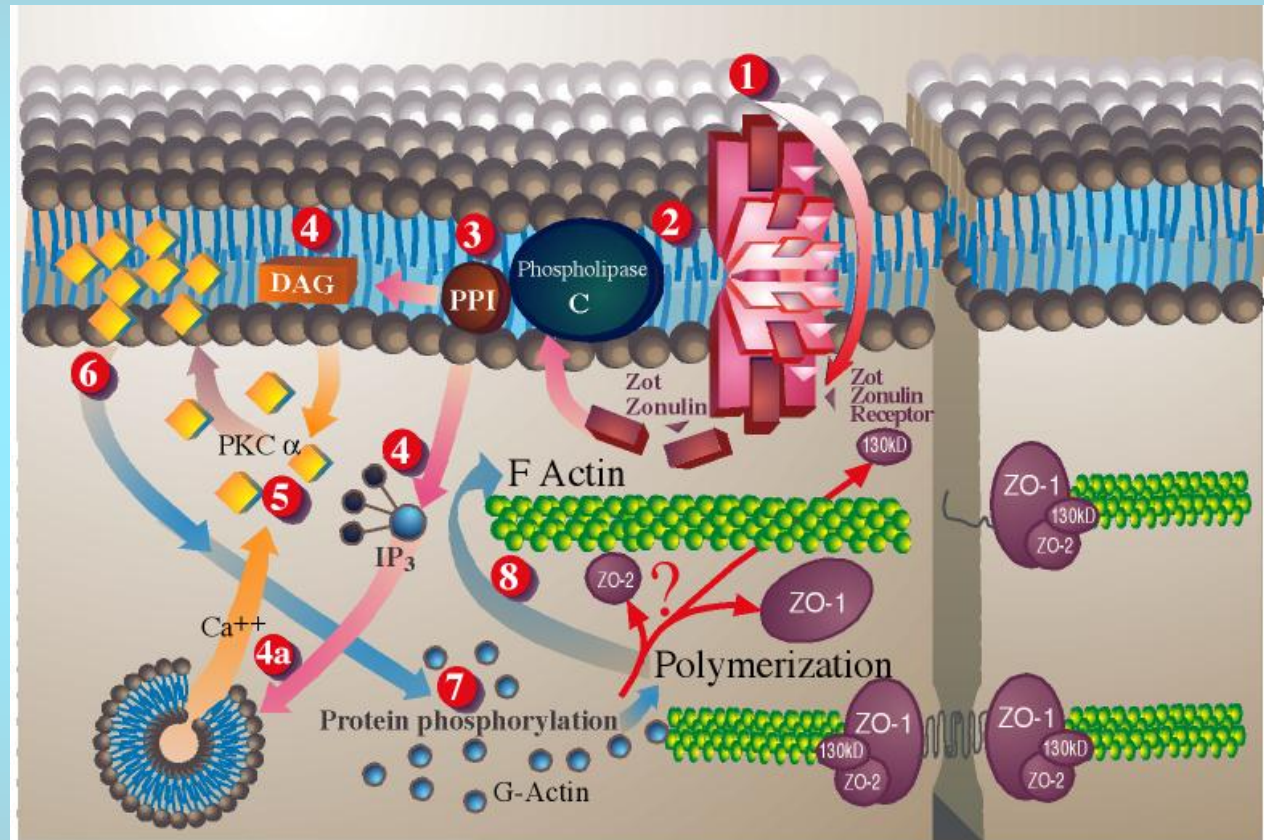
²Children's Hospital, Department of Medicine, Harvard Medical School, Boston, Massachusetts,

³Department of Microbiology and Immunology, University of Maryland School of Medicine.

¹Julie Brownley, ¹Ruiliang Lu, ¹Anna Sapone, ²Bao Lu, ²Craig Gerard, ^{1,3}Stefanie N. Vogel, ¹Alessio Fasano

The autoimmune process of Celiac Disease

- Triggered by consumption of gliadin
- Subsequent zonulin release
- Tight junctions of the small intestine become 'leaky'



Background

Finding: Gliadin effects on intestinal tight junctions and zonulin release are observed only when gliadin is added to the mucosal side of the membrane

Hypothesis: Gliadin may exert its effects on zonulin release and intestinal tight junctions by interacting with an intestinal luminal receptor

A gluten affinity column was designed to identify a potential brush border membrane receptor

- Soluble membrane preparations were obtained from rabbit small intestine
- Three proteins at 93, 100, and 107 kDa were eluted from the column

MALDI mass spec fingerprint analysis

ProFound - Search Result Details

Version 4.10.5
The Rockefeller University Edition

Details for rank 1 candidate in search BCF2DD6D-0518-4FA6E5DC

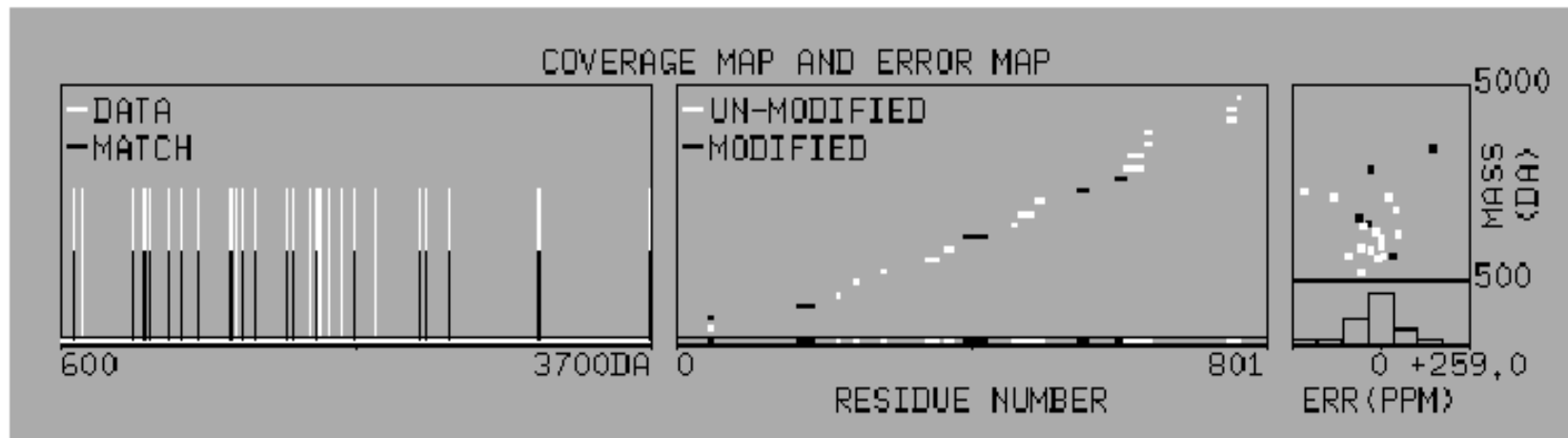
gi|20984919|ref|XP_125429.1| similar to interferon-inducible protein 10 (IP-10) receptor - mouse [Mus musculus]

Sample ID : R. Lu 100 KDa [Pass:0]

Measured peptides : 27

Matched peptides : 20

Min. sequence coverage: 32%

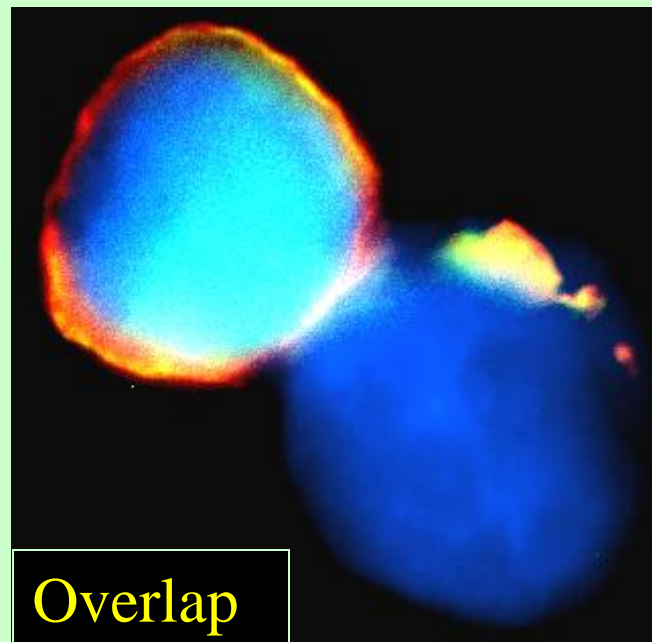
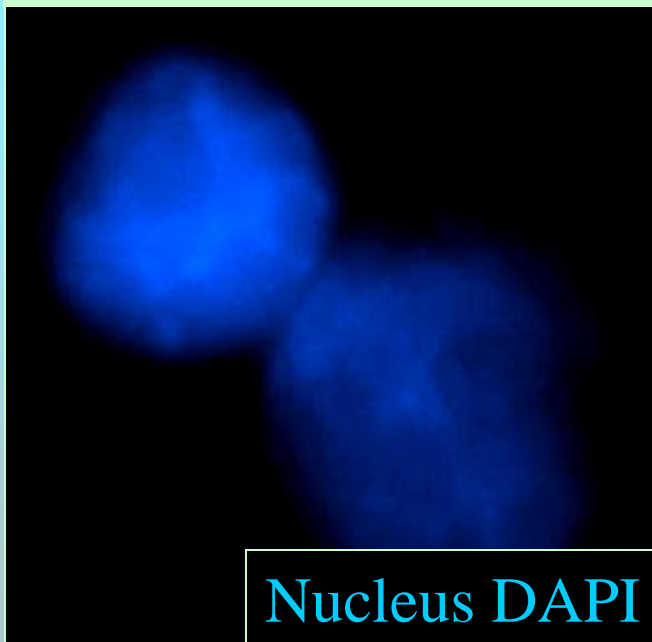
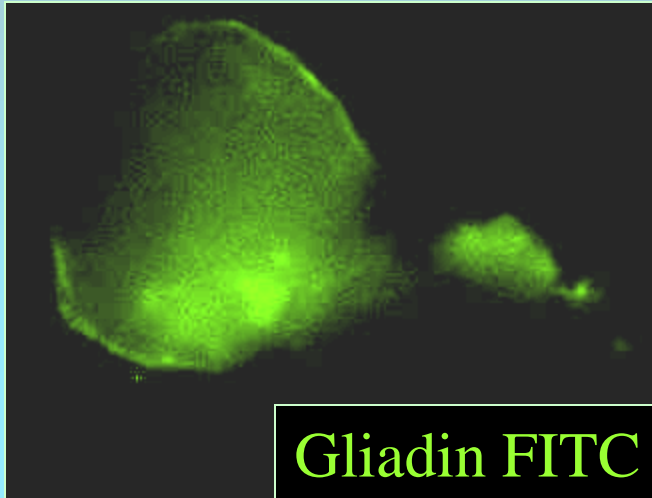


HEK293 cells were transfected with
CXCR3

visualize colocalization of gliadin and CXCR3
by immunofluorescence microscopy

CXCR3-Transfected HEK293 cells

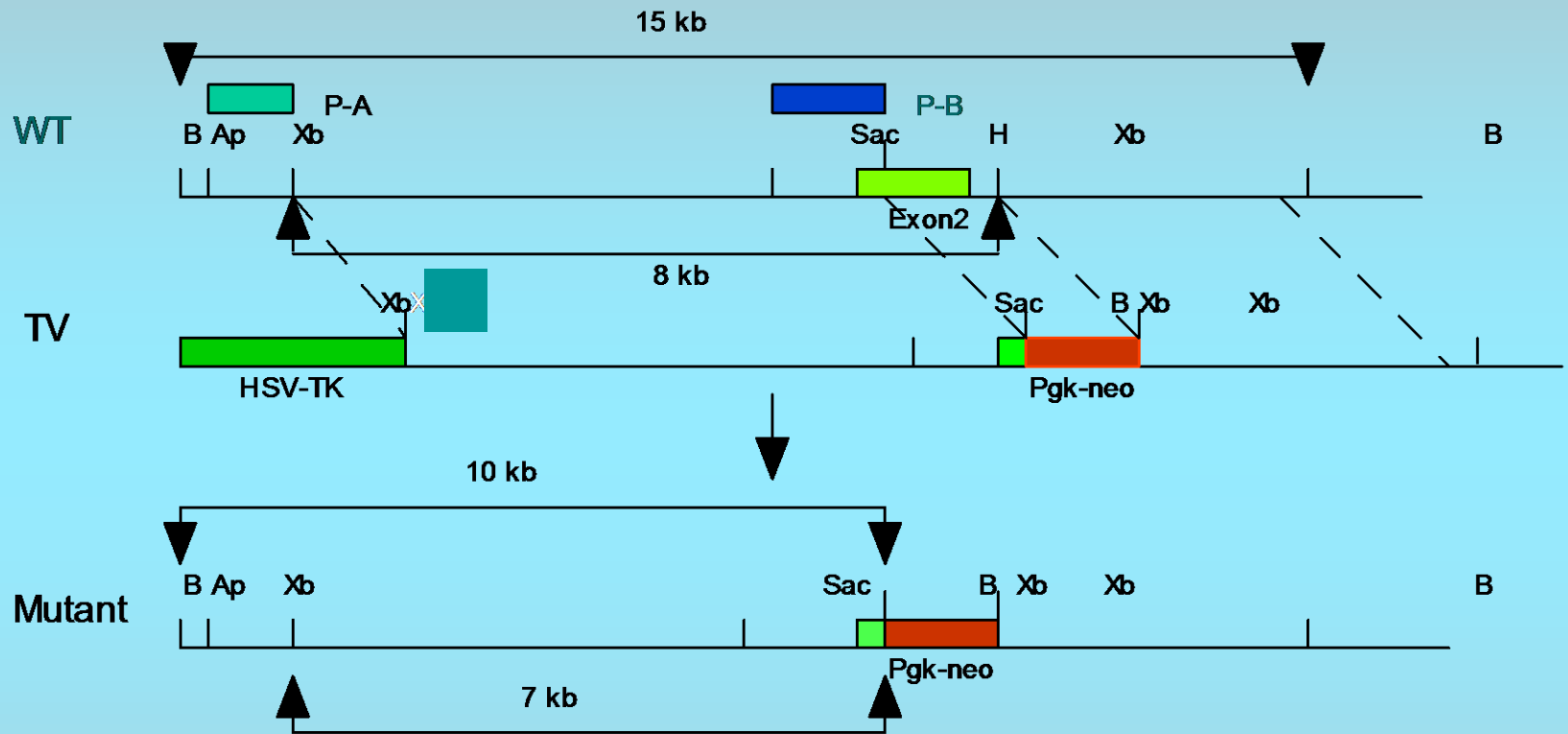
Stimulation with PT-gliadin [1 mg/ml]



To confirm the role of CXCR3 in gliadin induced zonulin release, intestinal permeability studies were performed using Wild type and CXCR3 knockout mice

CXCR3 Knockout Mice

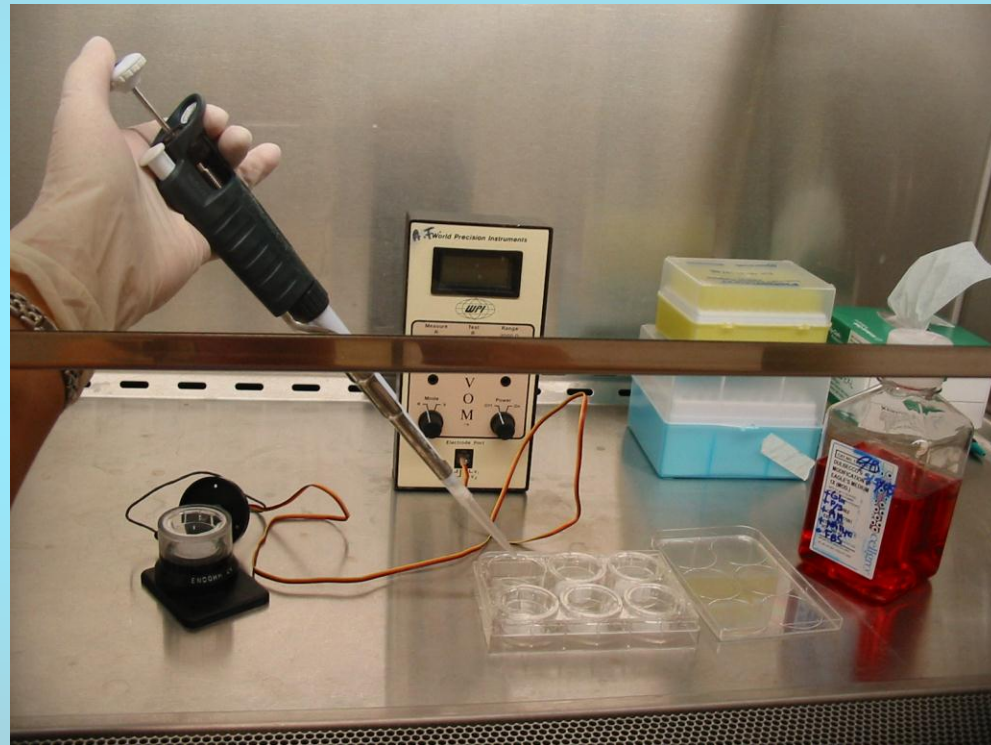
Homologous recombination into mCXCR3 Gene



	Expected Sizes kb	
	BamHI	XbaI
	P-A or P-B	P-B
WT	15	8
Mutant	10	7

Intestinal barrier function was assessed by transepithelial electrical resistance (TEER)

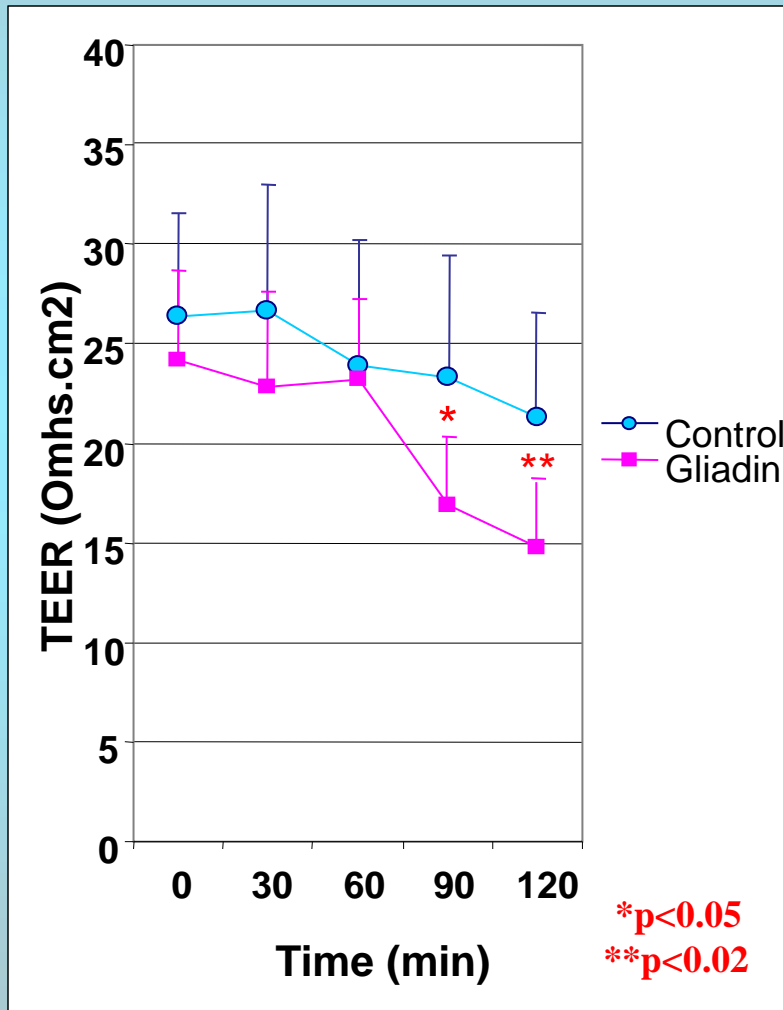
Micro-Snapwell System



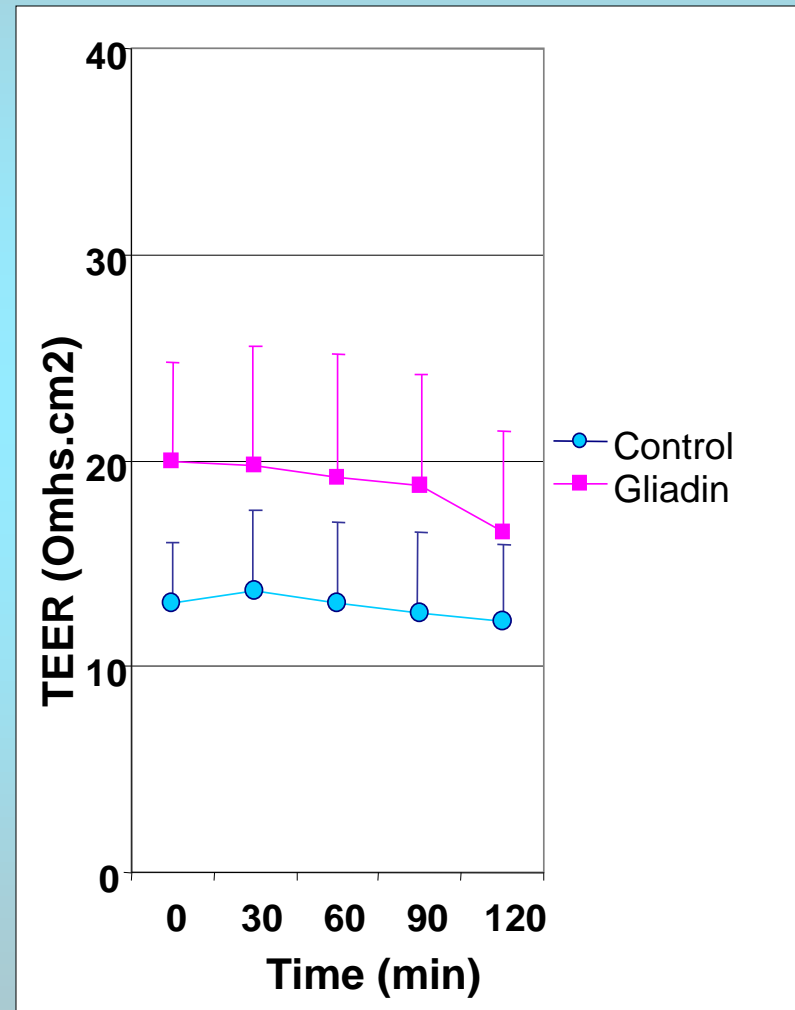
- Zonulin release was quantified by ELISA

Effect of PT-Gliadin on TEER

Wild Type Mice

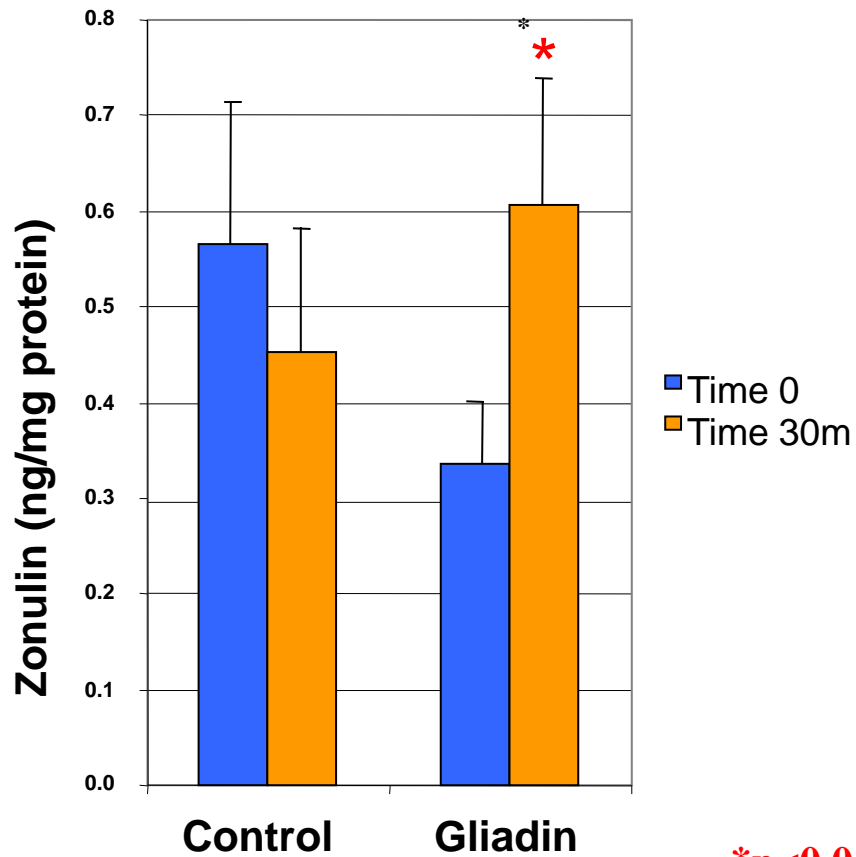


Knock Out Mice



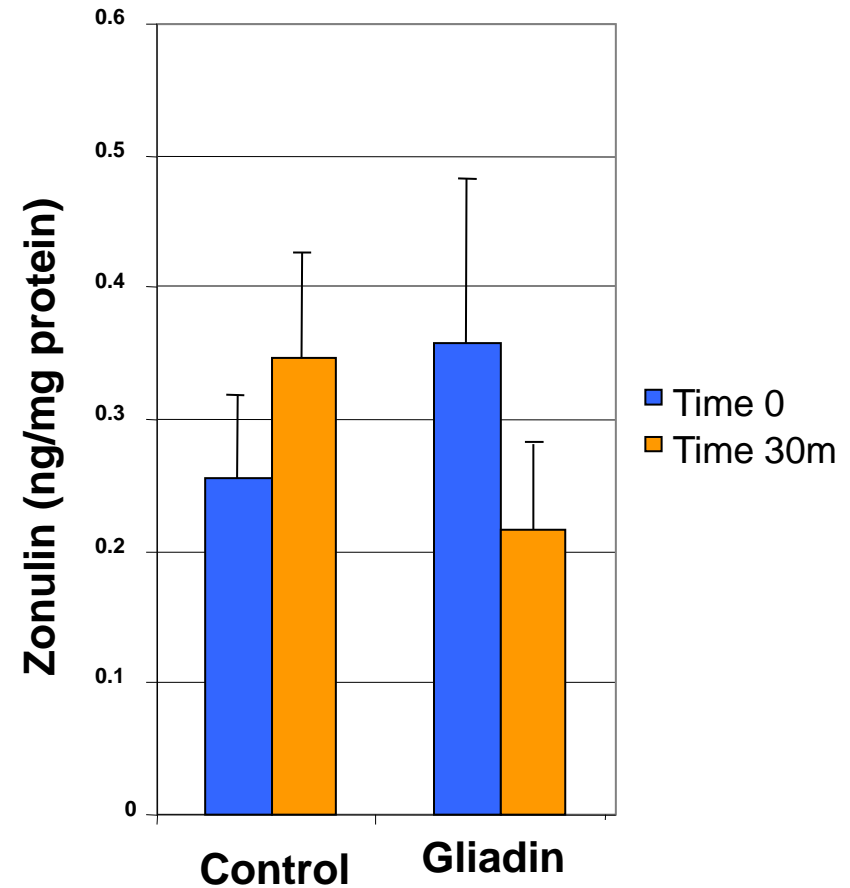
Effect of Gliadin on Zonulin Release

Control Mice

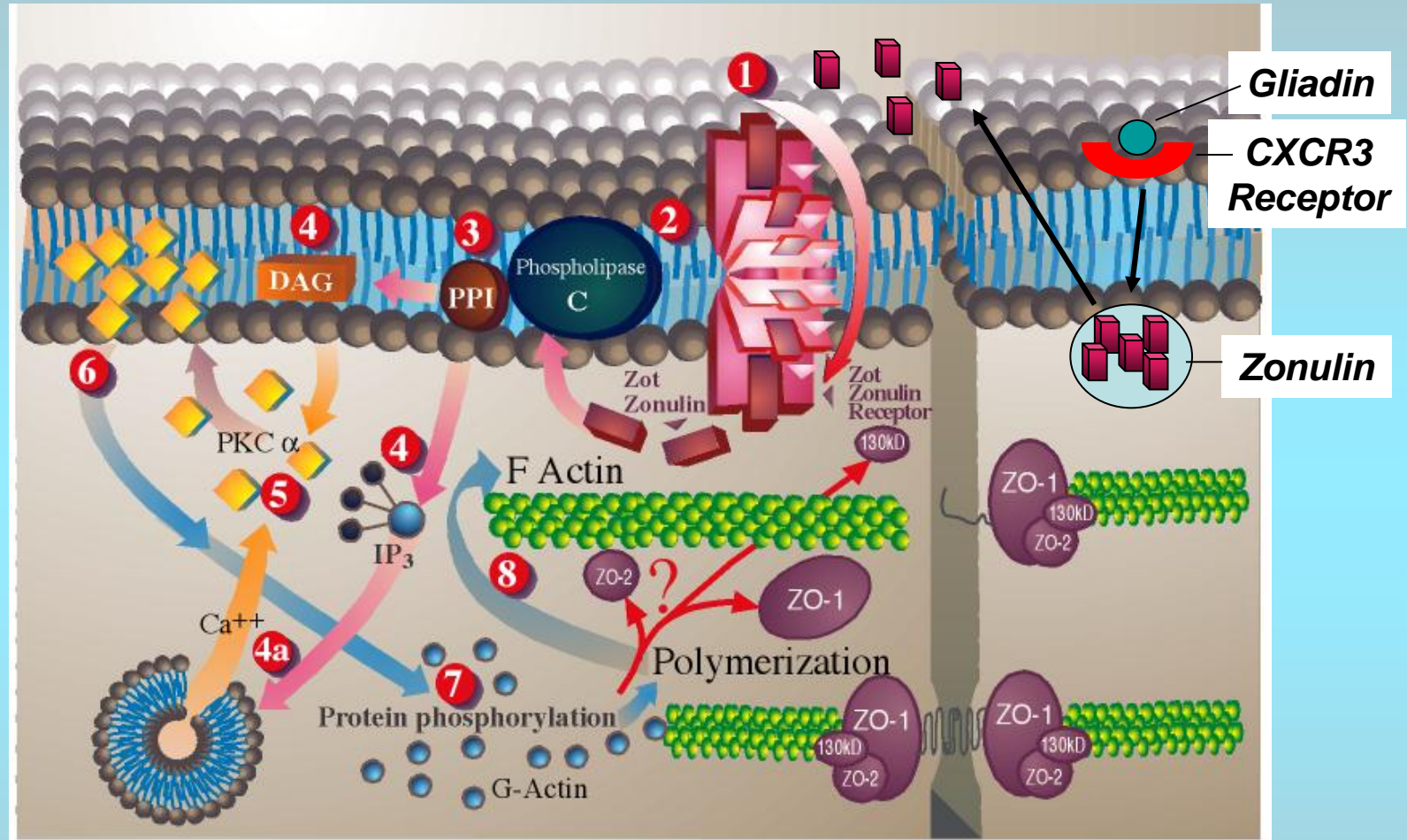


*p<0.05

CXCR3 Knockout Mice



Conclusions



The results from the affinity column, HEK293 transfection, and CXCR3 knockout animal studies suggest that CXCR3 is the intestinal receptor for Gliadin.