

Gliadin Binding to CXCR3 Causes Zonulin Release and Increased Intestinal Permeability

**Karen Lammers, Julie Brownley, Ruiliang Lu, Anna Sapone,
Bao Lu, Craig Gerard, Stefanie N. Vogel, Alessio Fasano**

**Mucosal Biology Research Center, Department of Pediatrics, and
Department of Microbiology and Immunology,
University of Maryland School of Medicine, and
Children's Hospital, Department of Medicine,
Harvard Medical School, Boston, Massachusetts**

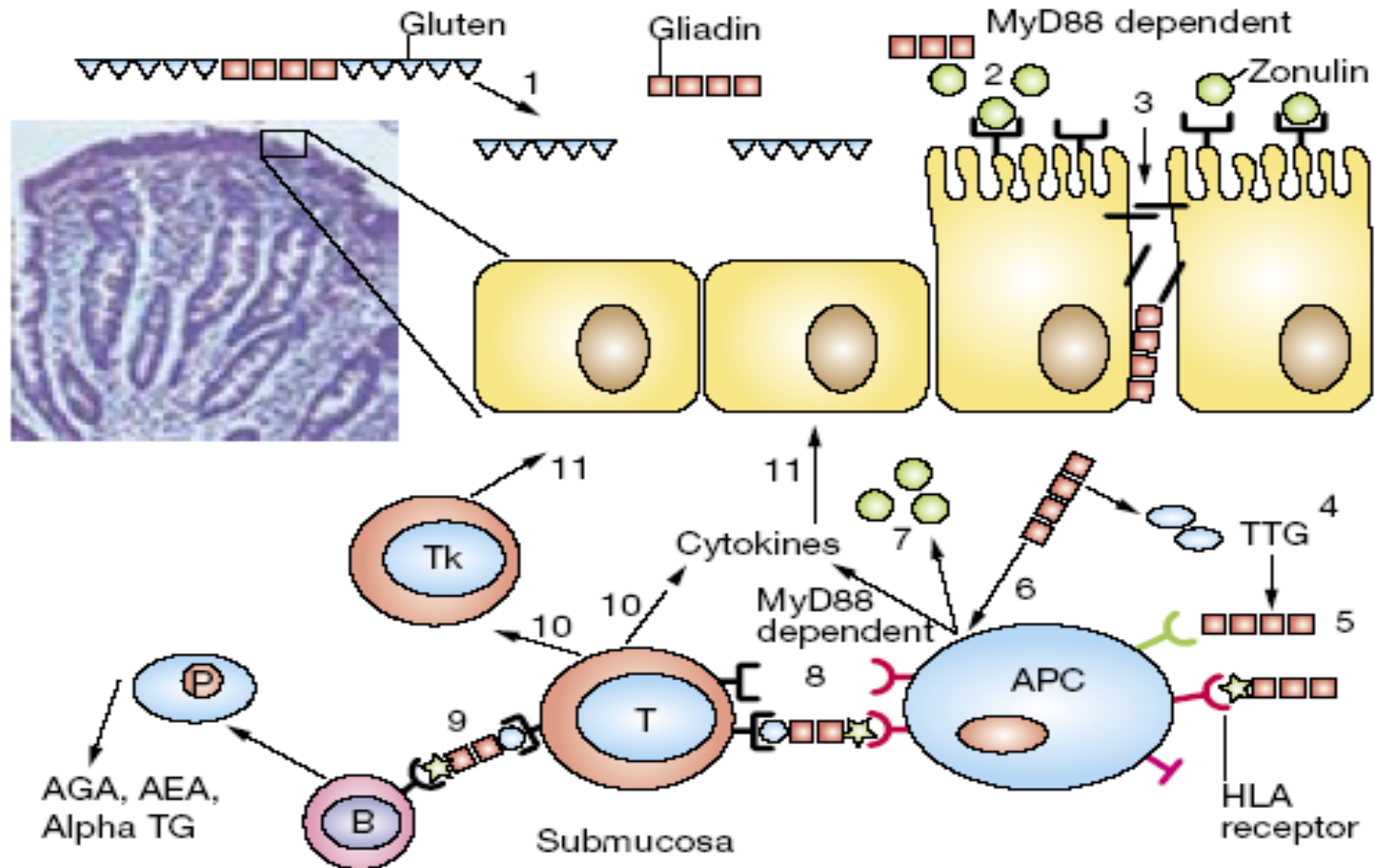


Background

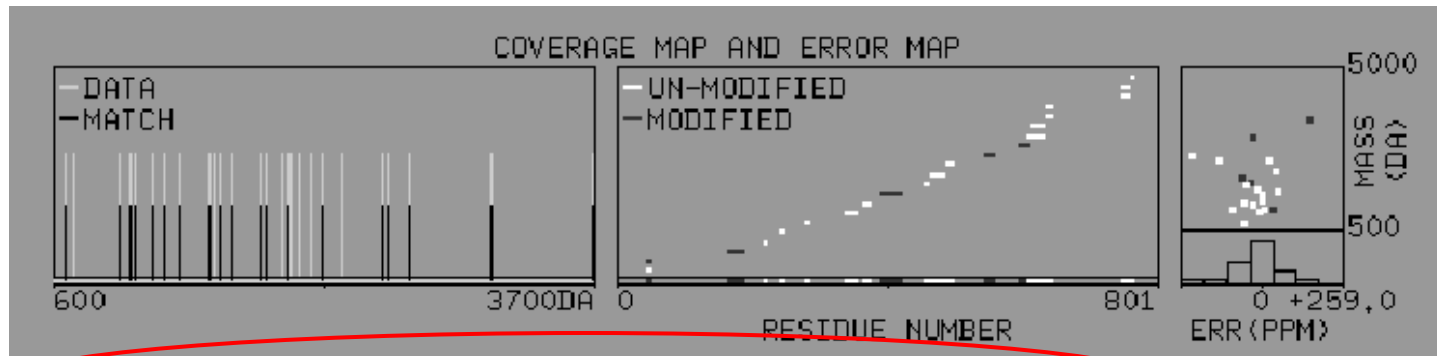
- **Celiac disease is an immune-mediated enteropathy triggered by the ingestion of gluten-containing grains;**
- **Celiac disease is unique since it is the only autoimmune disease for which the triggering environmental factor, gliadin, is known;**
- **Gliadin induces the release of zonulin (an intestinal protein involved in the regulation of intestinal permeability) that initiates the onset of the autoimmune process;**
- **Epithelial zonulin release and the parallel increase in intestinal permeability occur after apical, but not basolateral, exposure to gliadin**

AIM

To identify the gliadin intestinal epithelial apical receptor involved in zonulin release and loss in intestinal barrier function



Gliadin Affinity Column Results: Gliadin Receptor Binding

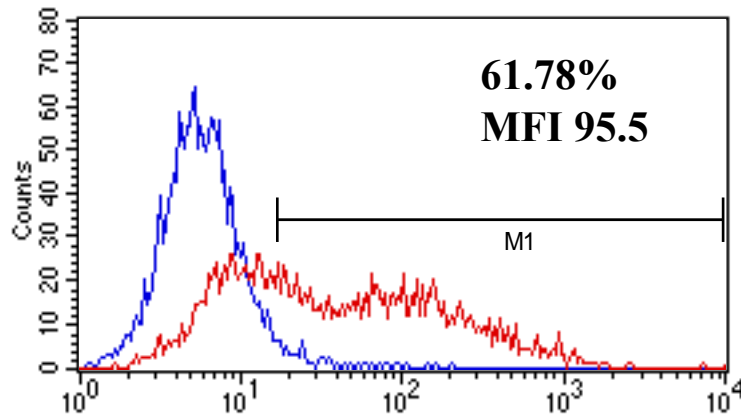
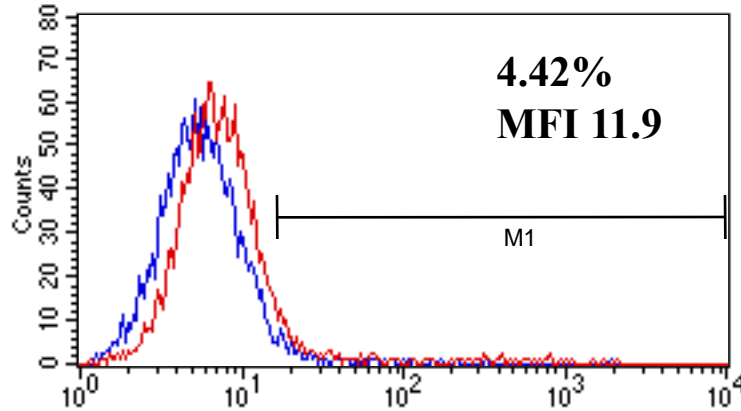


similar to interferon-inducible protein 10 (IP-10, CXCR3) receptor - mouse

Measured Mass(M)	Avg/ Mono	Computed Mass	Error (ppm)	Residues Start To	Missed Cut	Peptide sequence
669.289	M	669.323	-51	762 767	0	GFGSFR
985.519	M	985.526	-7	218 225	0	EMVELPLR
1050.525	M	1050.516	8	46 53	0	MDELQLFR
1066.557	M	1066.511	43	46 53	0	MDELQLFR
1074.430	M	1074.530	-93	278 287	0	LAGESESNLR
1171.628	M	1171.659	-27	240 251	0	GILLYGPPGTGK
1241.561	M	1241.625	-51	366 377	0	EVDIGIPDATGR
1328.689	M	1328.683	5	454 465	0	WALSQSNPSALR
1500.708	M	1500.702	4	750 761	0	YEMFAQTLQQSR
1555.931	M	1555.849	53	634 646	0	LDQLIYIPLPDEK
1628.774	M	1628.797	-14	749 761	1	KYEMFAQTLQQSR
1798.892	M	1798.982	-50	634 648	1	LDQLIYIPLPDEKSR
1822.855	M	1822.916	-33	595 610	1	VINQILTEMDGMSTKK
1822.855	M	1822.909	-30	488 502	0	ELQELVQYPVEHPDK
1950.794	M	1950.914	-61	544 560	0	GPELLTMWFGSEANVR
2145.247	M	2145.147	47	339 358	0	AHVIVMAATNRPN SIDPALR
2497.328	M	2497.269	24	466 487	1	ETVVVEVPQVTWEDIGGLEDVKR
2518.616	A	2518.945	-131	611 633	0	NVFIIGATNRPD IIDPAILRPGR
2646.547	A	2647.119	-216	610 633	1	KNVFIIGATNRPD IIDPAILRPGR
3110.487	A	3110.559	-23	165 191	1	VVETDPSPYCIVAPD TVIHCEGEPIKR
3688.625	A	3688.047	157	390 424	0	LADDVDLEQVANETHGHV GADLAALCSEALQAIR

Gliadin Binds to CXCR3

CXCR3-transfected HEK293T cells express CXCR3

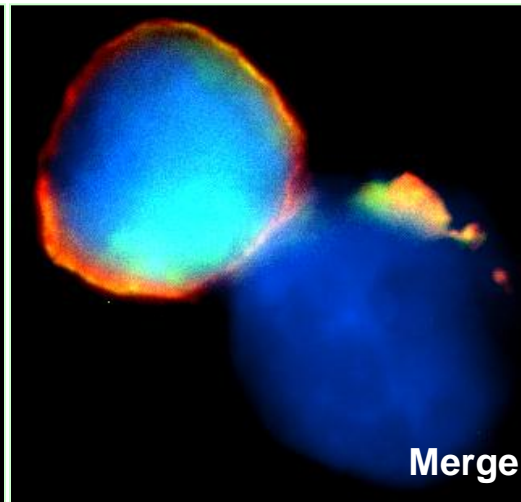
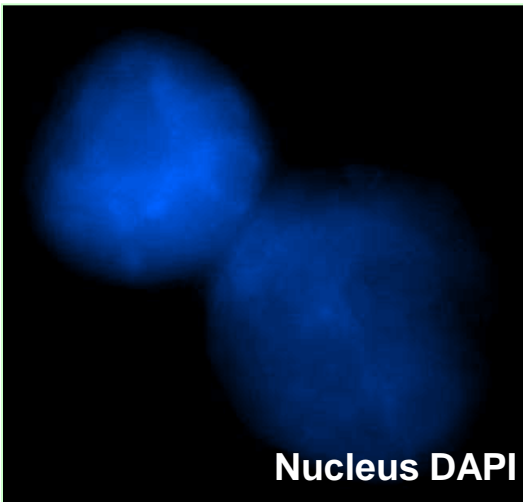
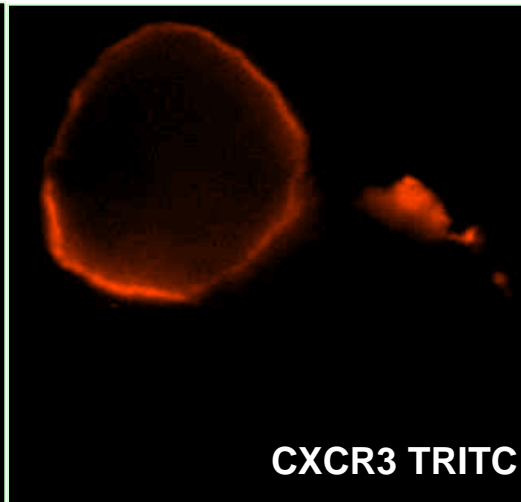
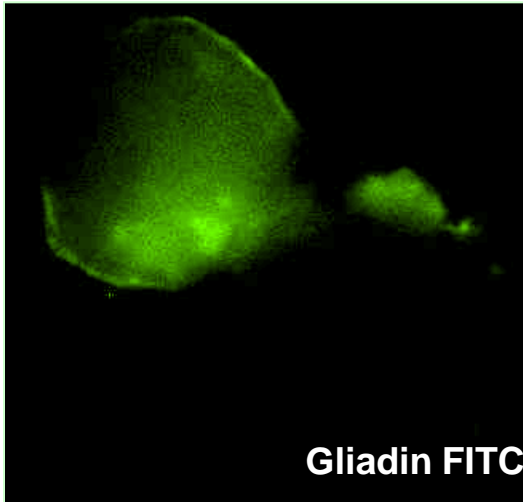


Gliadin Binds to CXCR3

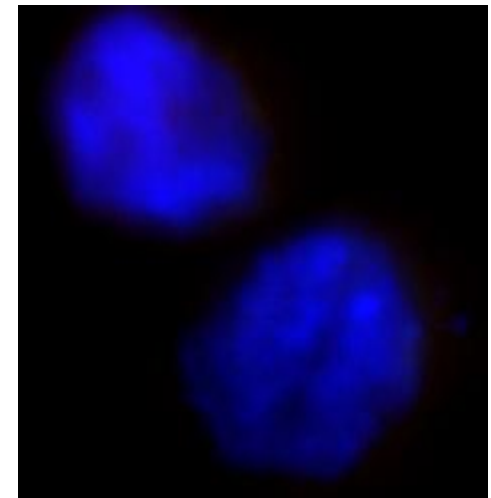
Co-localization of PT-gliadin and CXCR3 in CXCR3-transfected HEK293 T cells:

Cells transfected with vector + CXCR3

Cells transfected with vector alone



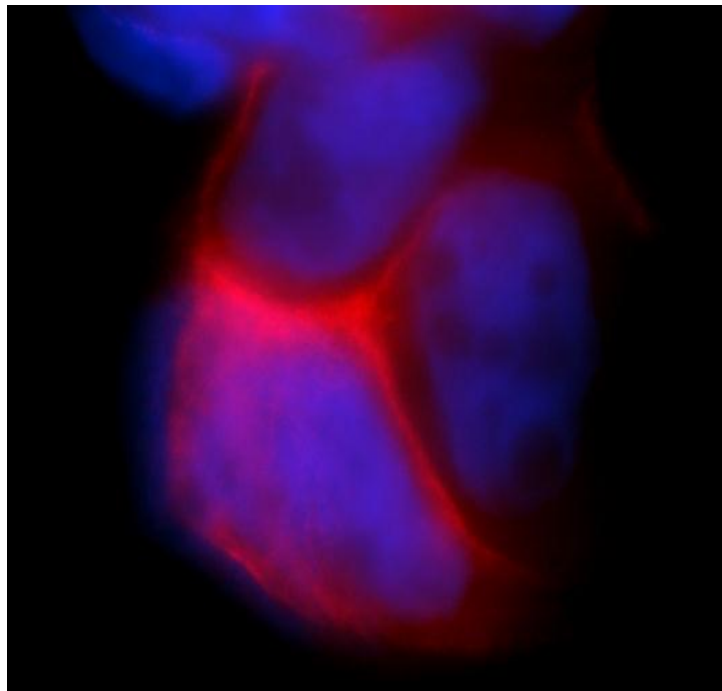
Magnification 100x



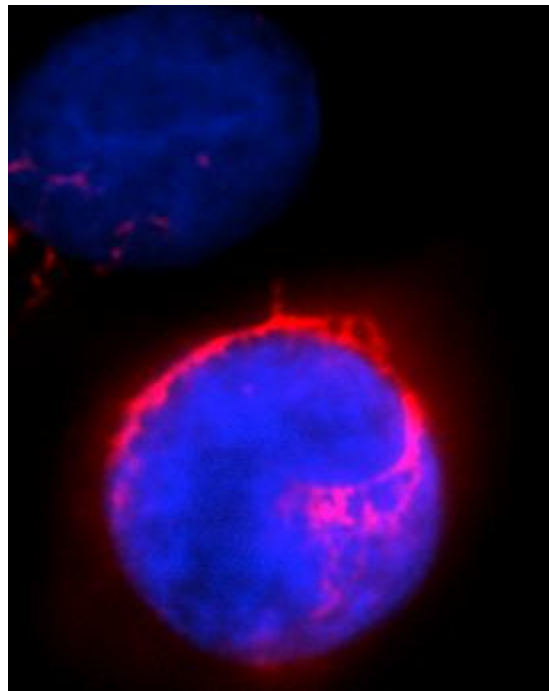
Magnification 100x

CXCR3 is Expressed on Intestinal Epithelial Cells

CXCR3 expression in intestinal epithelial cell lines



CaCo-2

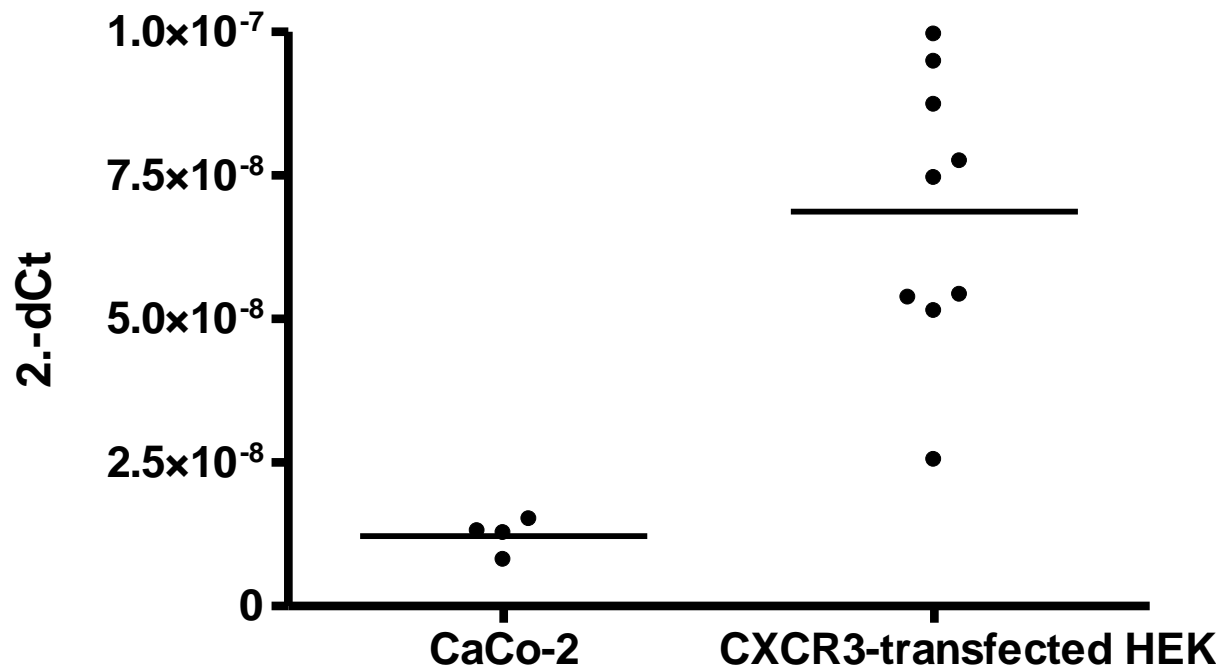


IEC6

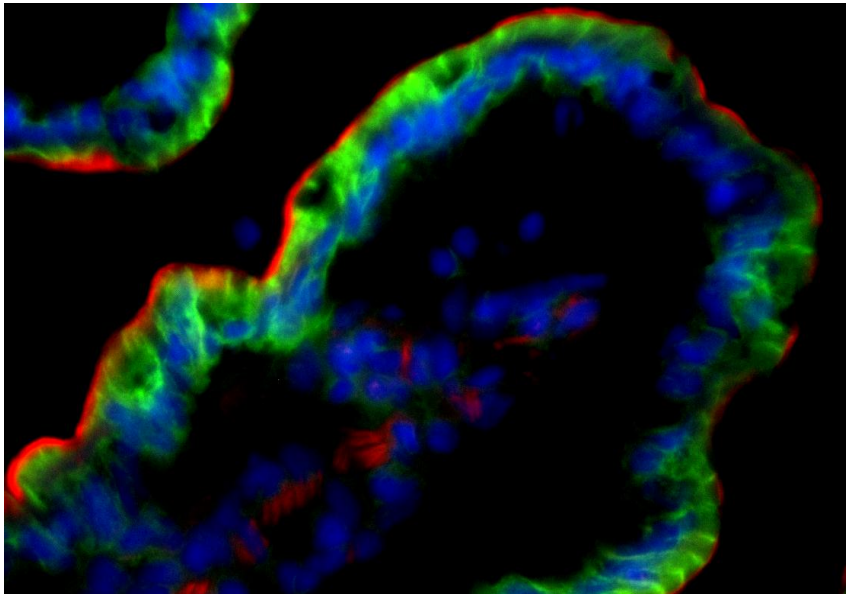
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CXCR3 is Expressed on Intestinal Epithelial Cells

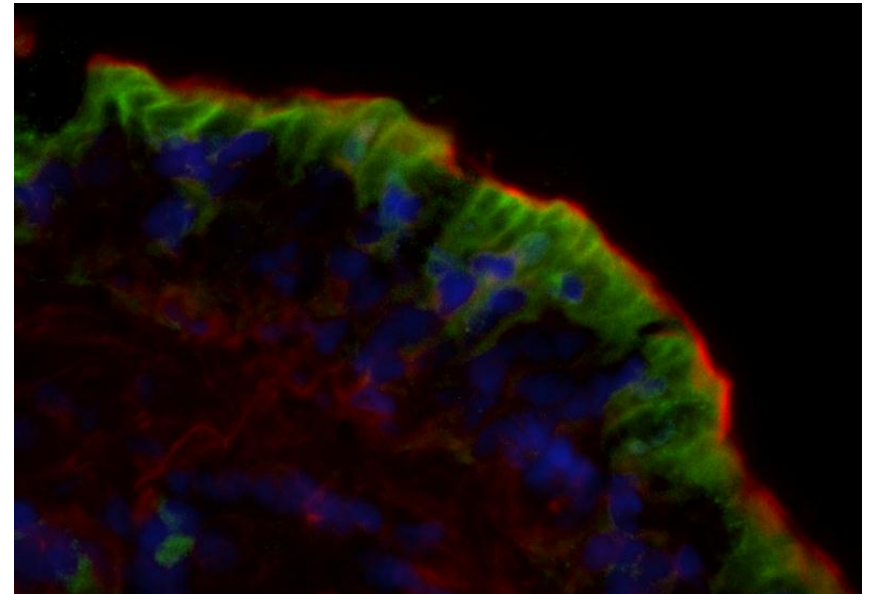
CXCR3 mRNA expression in CaCo-2 cells and CXCR3-transfected HEK293T cells



CXCR3 is Expressed on The Enterocytes Brush Border and in the Intestinal Lamina Propria



40x



100x

Red: CXCR3

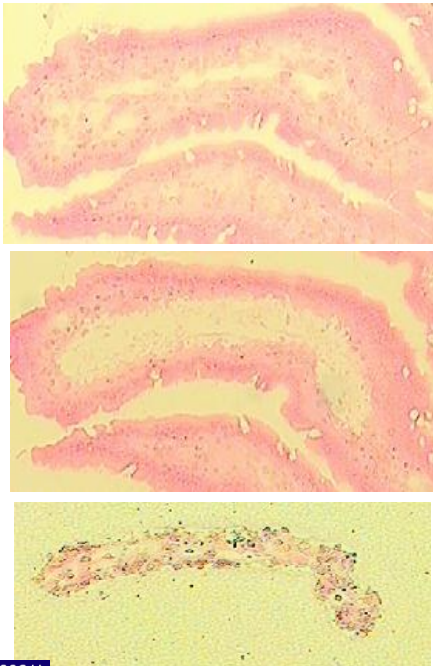
Green: Cytoskeleton

Blue: Nuclei

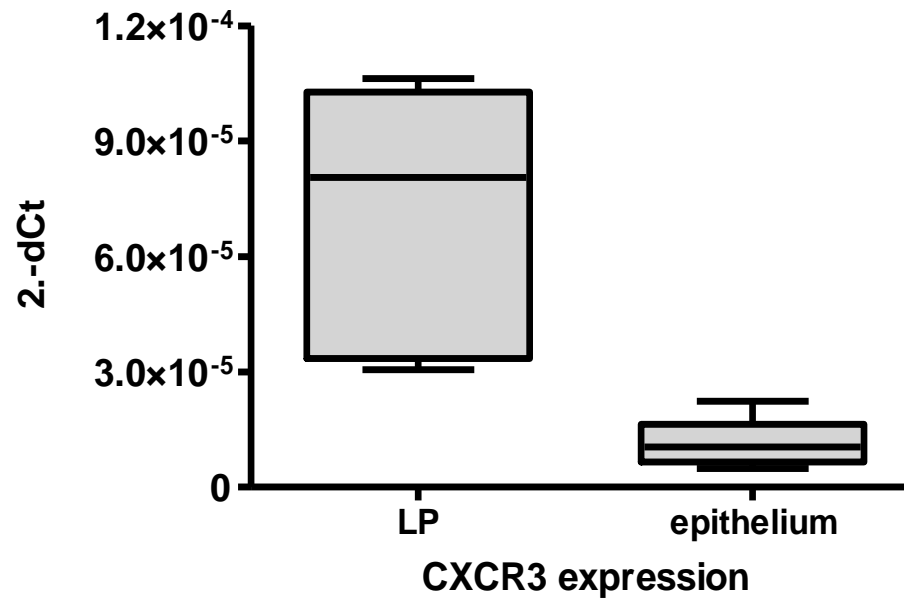
CXCR3 is Expressed on Intestinal Epithelial Cells

Laser capture microdissection of mouse intestinal tissue: CXCR3 mRNA expression

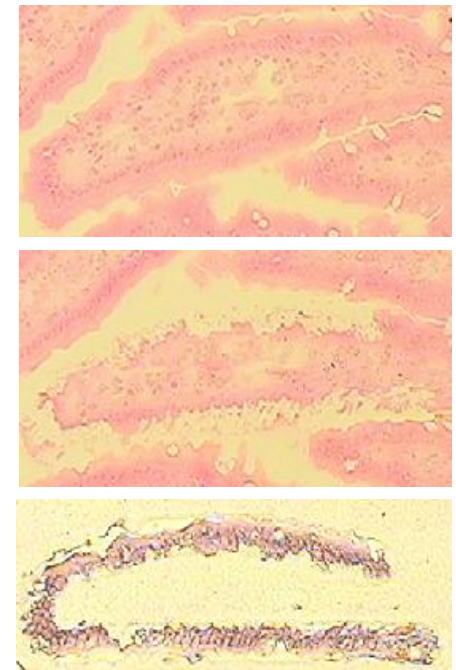
Laser Capture
Lamina propria preparation



Real Time
RT-PCR

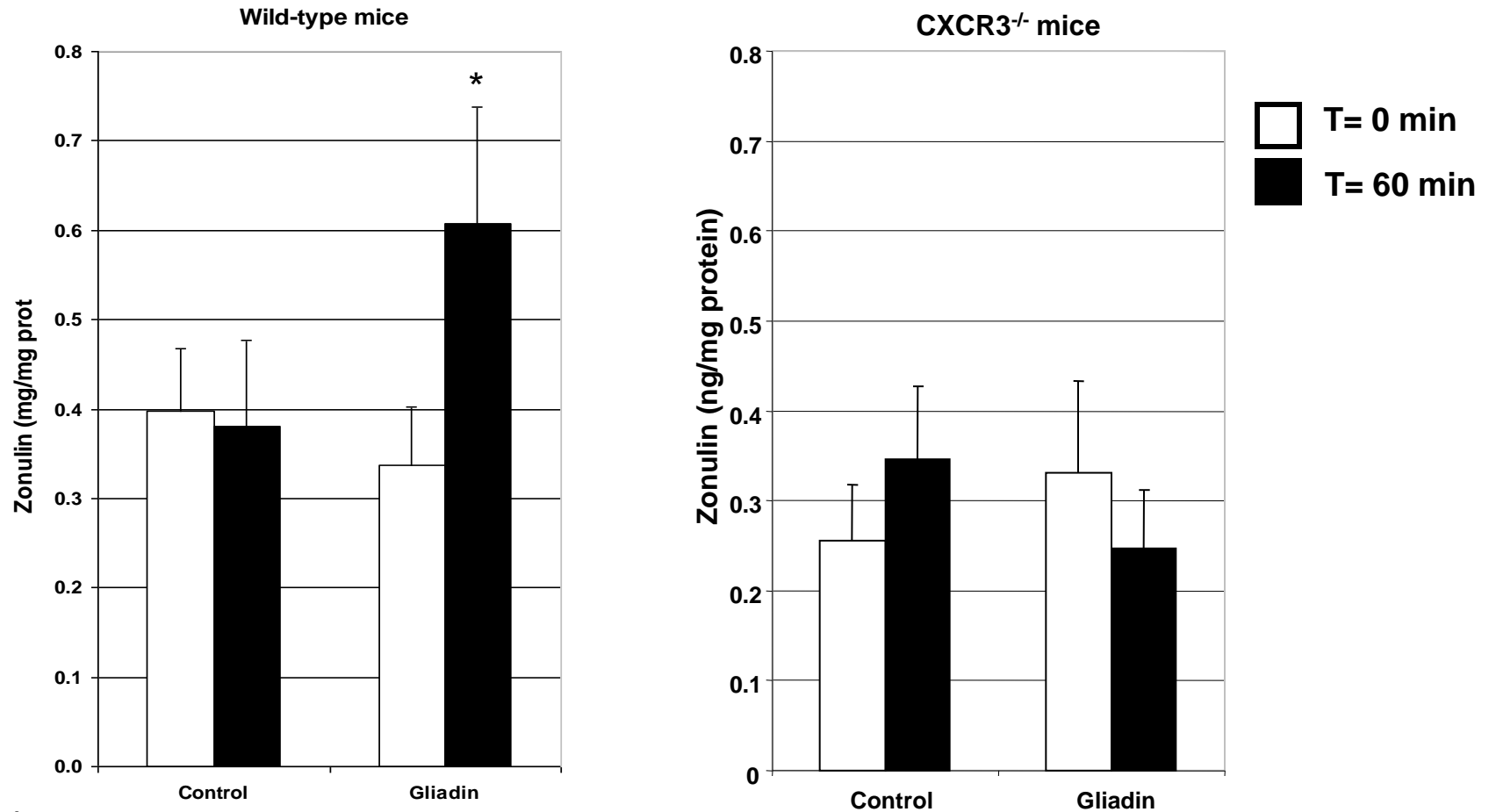


Laser Capture
Epithelial tissue preparation



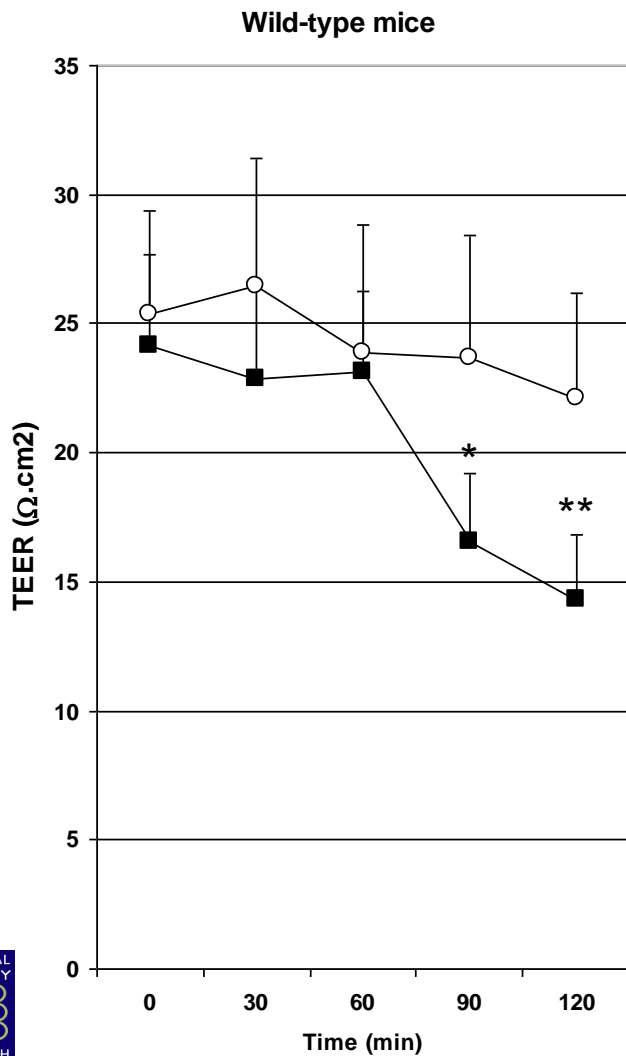
CXCR3^{-/-} Mice do not Release Zonulin Following Intestinal Challenge with PT-gliadin

PT-gliadin induces increased zonulin release in wild-type but not CXCR3^{-/-} mice

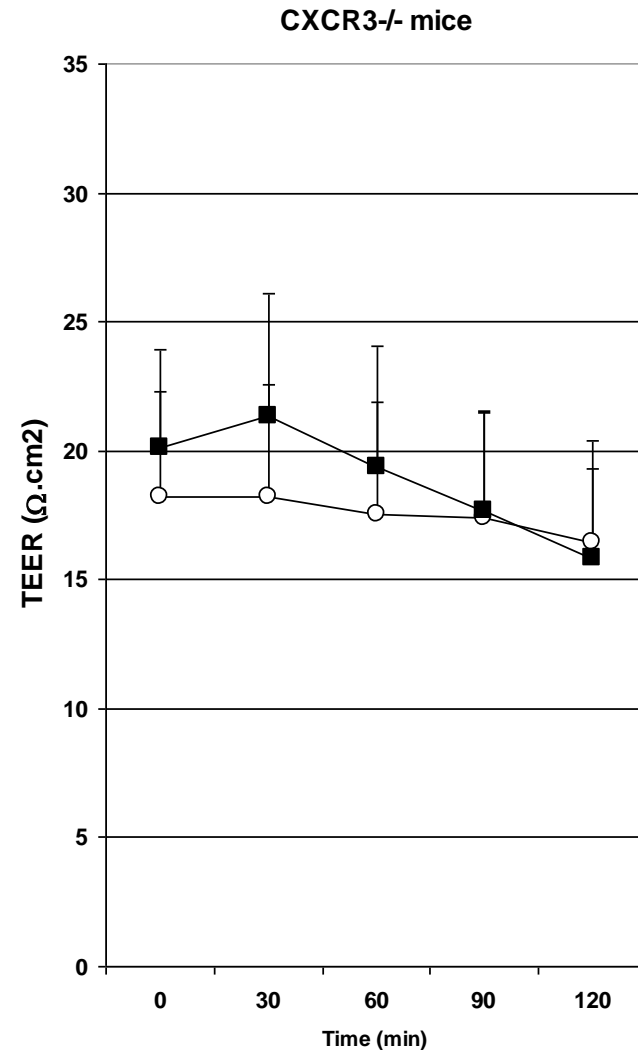


*p < 0.05

PT-gliadin Induces TEER Changes in Wild-type but not CXCR3^{-/-} mice

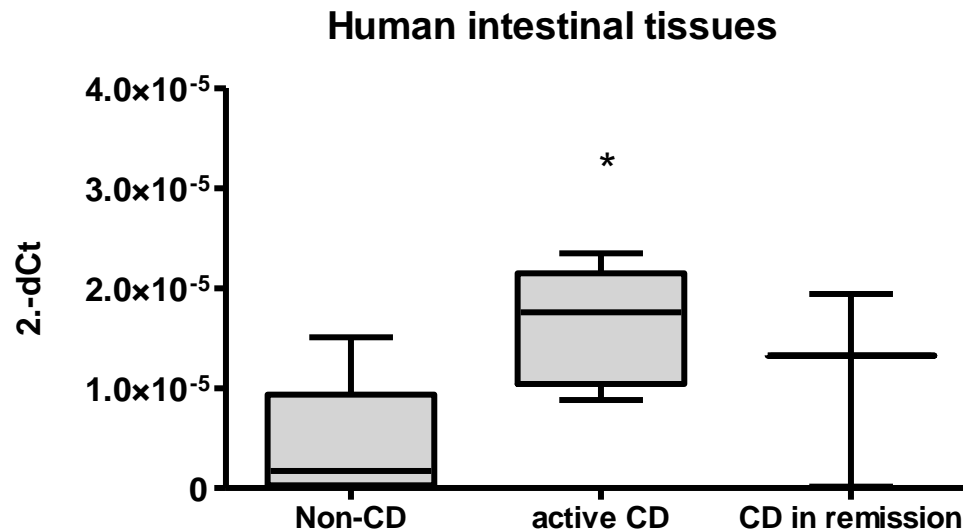


*p<0.05
**p<0.02



CXCR3 Expression in Human Intestinal Tissue is Up-Regulated by Gliadin Exposure in Celiac Disease (CD) Patients

CXCR3 mRNA expression in human intestinal tissues



*p=0.004

Conclusions

- **The chemokine receptor CXCR3 is the gliadin intestinal mucosal receptor that leads to activation of the zonulin pathway;**
- **CXCR3 is expressed on the surface of enterocytes;**
- **CXCR3 is over-expressed in celiac disease patients when ingesting gluten and returns to baseline expression when patients embrace a gluten free diet.**