

Periostin Modulates fMLP-directed Neutrophil Migration in Intestinal Inflammation

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Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is an inflammatory condition that affects the gastrointestinal tract and is commonly diagnosed as Crohn's Disease (entire GI tract, terminal ileum and large intestine) and Ulcerative Colitis (large intestine and rectum)

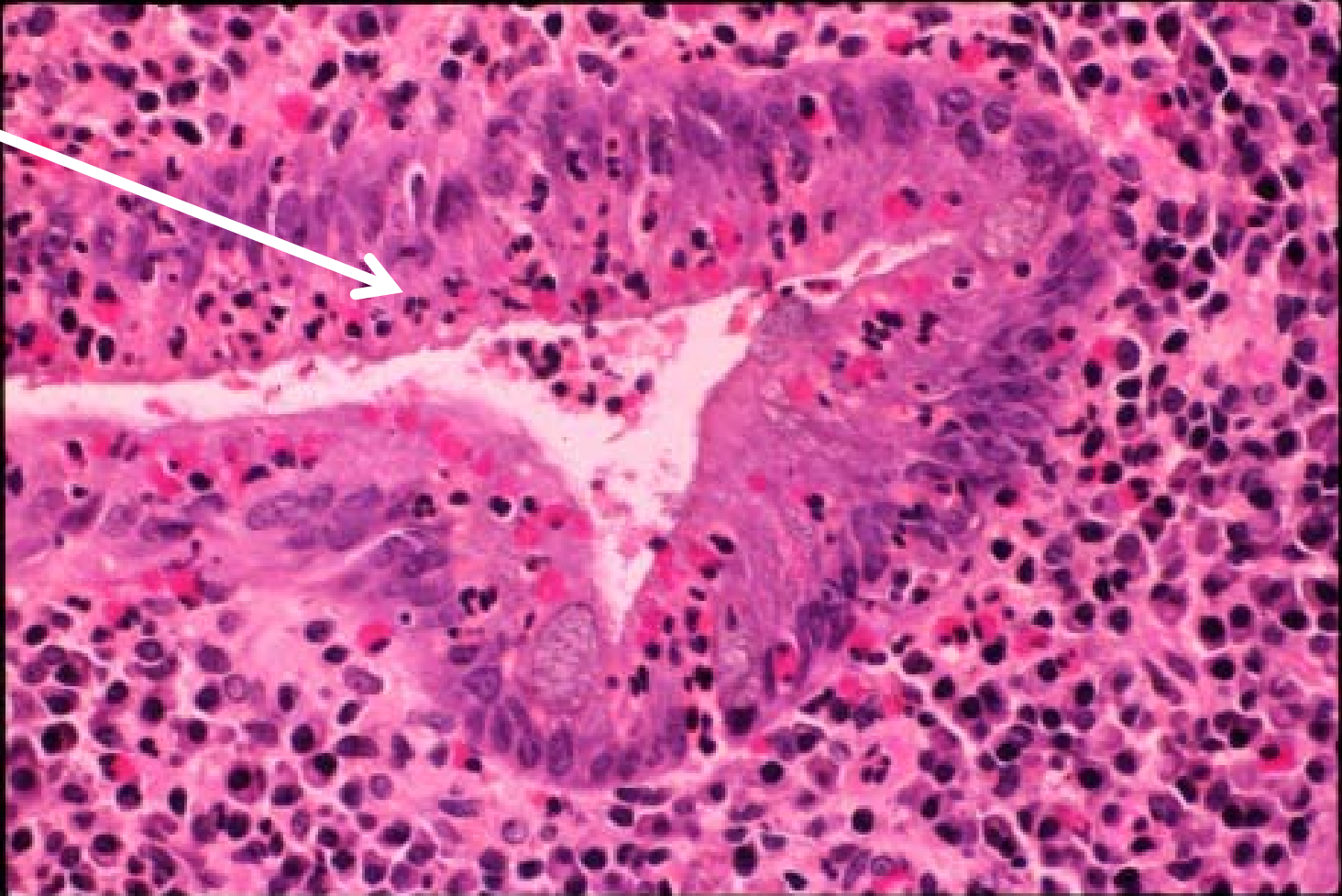
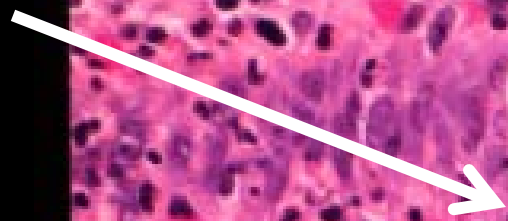
The features include neutrophil (PMN) migration toward the intestinal lumen; aberrant cytokine production (e.g., Interleukin-6 (IL-6)); epithelial destruction; and over activation of intestinal fibroblasts

PMN migration across the intestinal epithelium is a key feature in both Crohn's Disease and Ulcerative Colitis

These observations strongly suggests that PMN migration across the intestinal epithelium plays a central causative role in the disease process

Ulcerative Colitis

PMN



Periostin

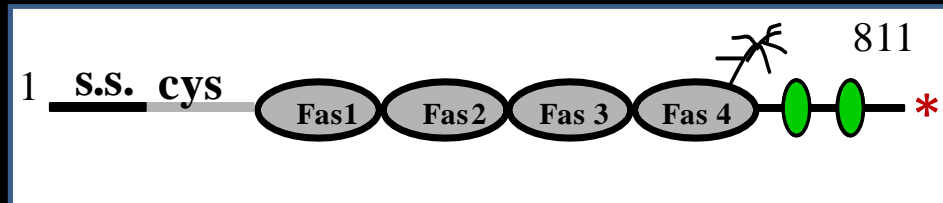
ECM 90-kDa protein expressed in connective tissue that includes tendons, skin, bone, heart valves (diseased) and cancerous tissue

Periostin is produced by fibroblasts, up regulated during cellular injury (i.e., early inflammation), and appears to be involved in the modulation of the characteristic transition from fibroblast to myofibroblast

One publication has shown that in the intestine periostin is present in close proximity to the sub-epithelial fibroblasts

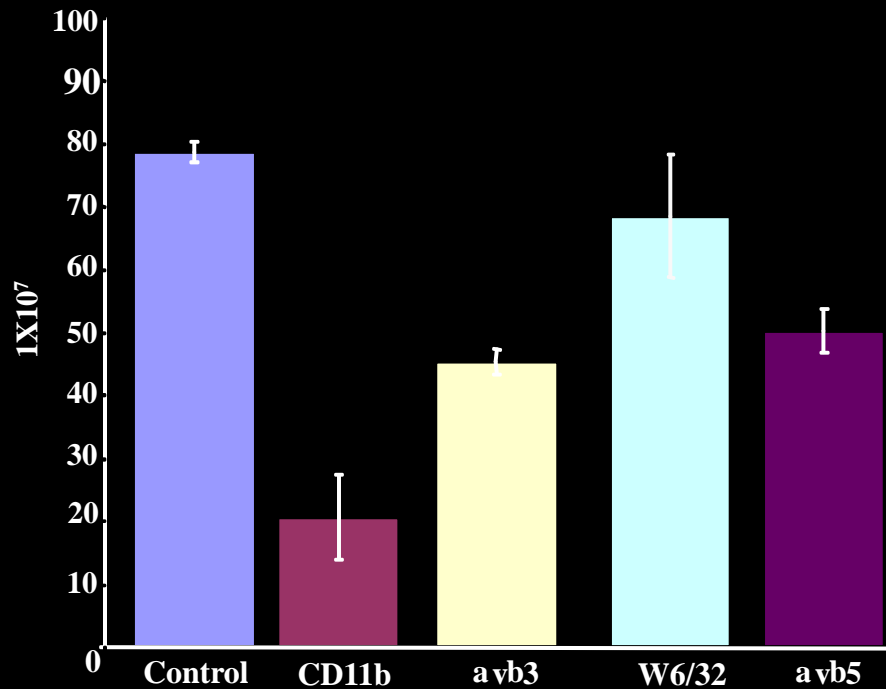
A role for periostin in neutrophil (PMN) transmigration or expression in the intestine has not been explored

Schematic of Periostin Domain Architecture

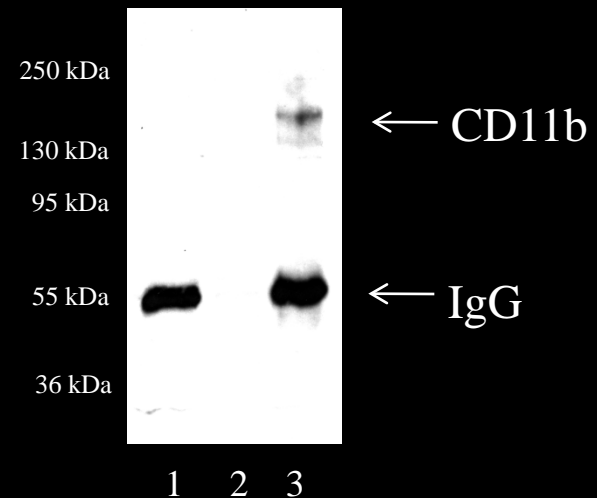


Human PMN attach robustly to Periostin

Periostin is linked to CD11b localized on PMN



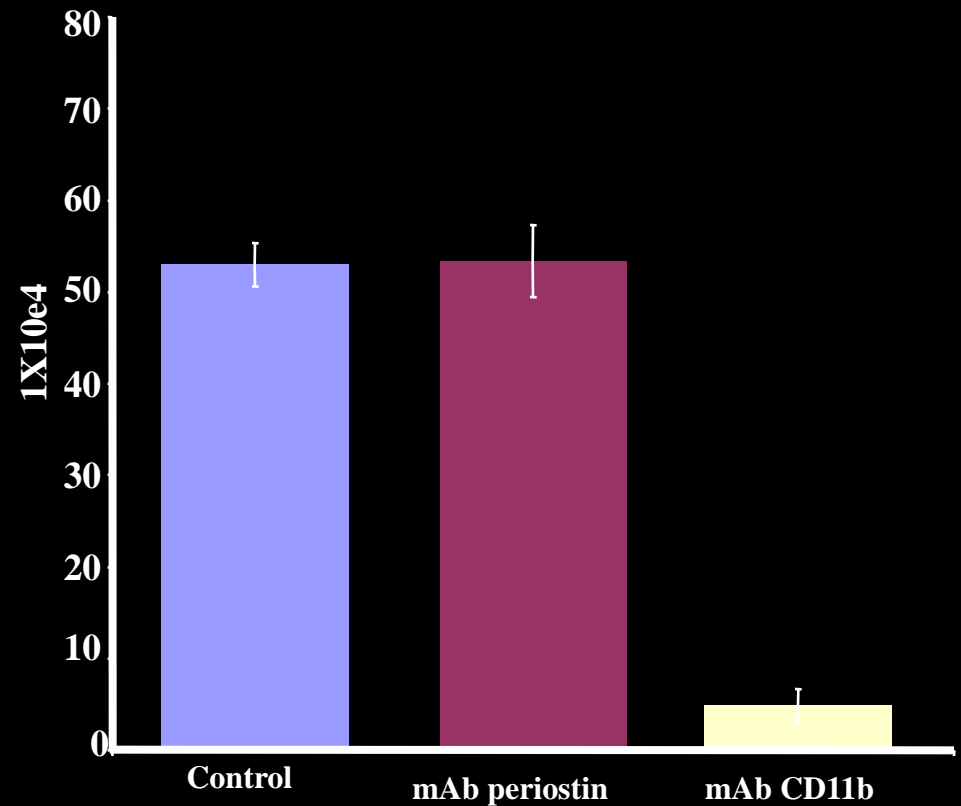
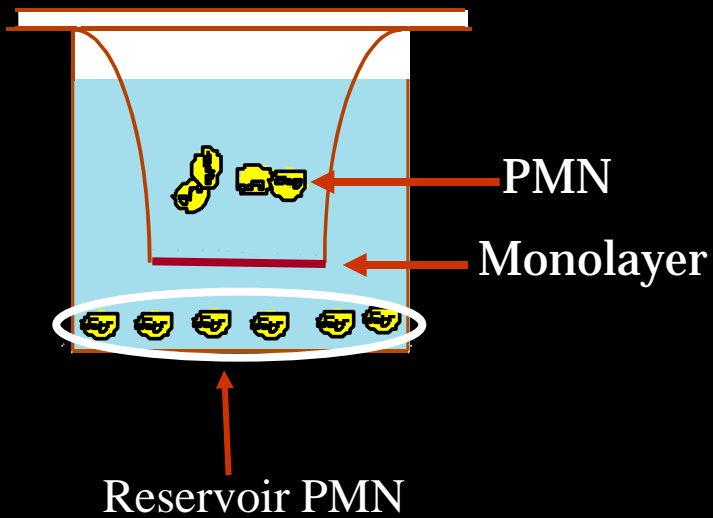
CD11b inhibits PMN attachment to purified periostin better than established periostin receptors



1. Post bind
2. Wash
3. IgG beads

Immunoprecipitation of CD11b (170kDa) from PMN using periostin antibodies

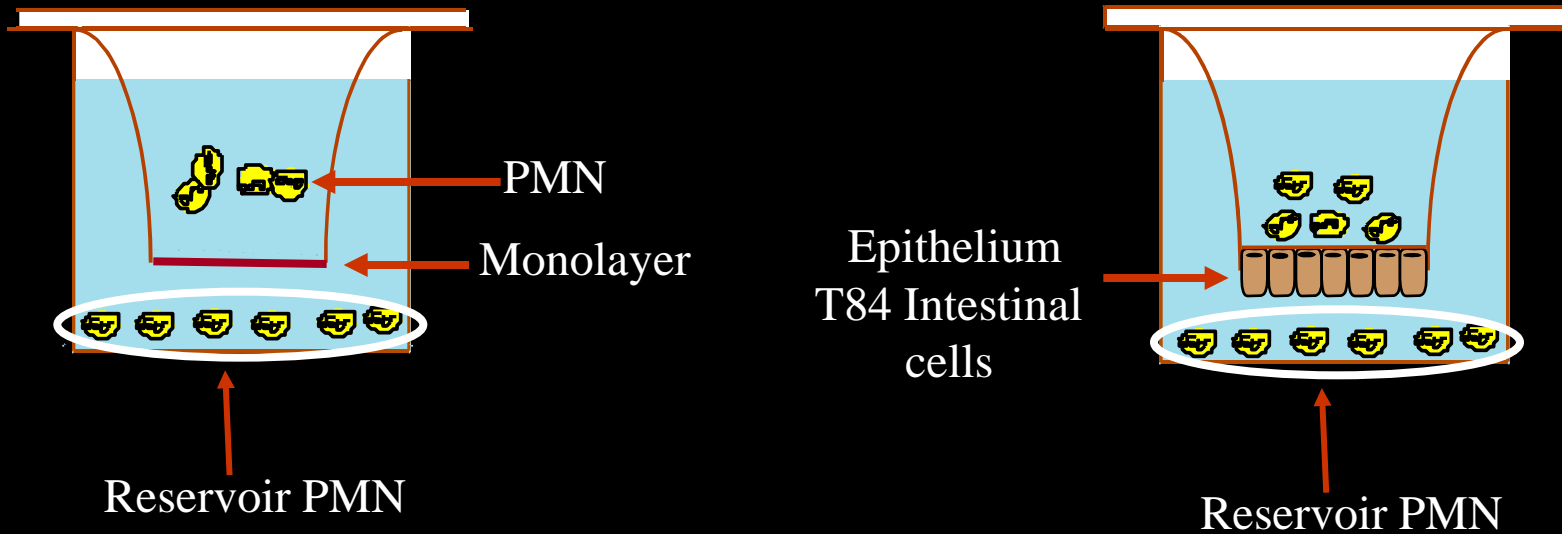
Anti-periostin antibodies do not inhibit PMN transfilter migration



Purified periostin does not affect PMN transfilter migration

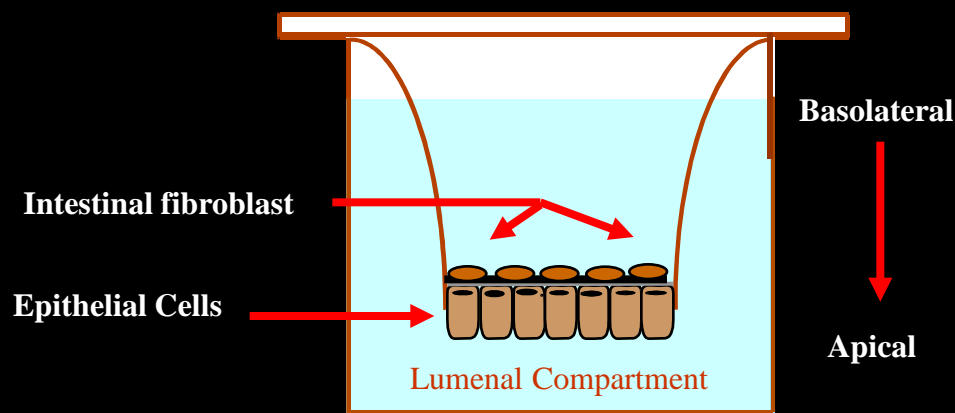
PMN Transmigration

Numerous studies have examined PMN transmigration across collagen coated monolayers and/or polarized epithelial cells

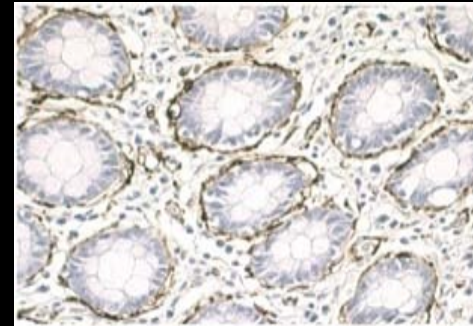


Few studies have investigated the role for intestinal fibroblast in PMN transmigration

PMN Transmigration: Intestinal Fibroblasts and Epithelial Cells *in vivo* configuration



Human Large Intestine



Brown: indicates smooth muscle actin and represents intestinal fibroblast

Resistance Measurements: T84 Epithelial Cells

Cells Measured as Ω cm²

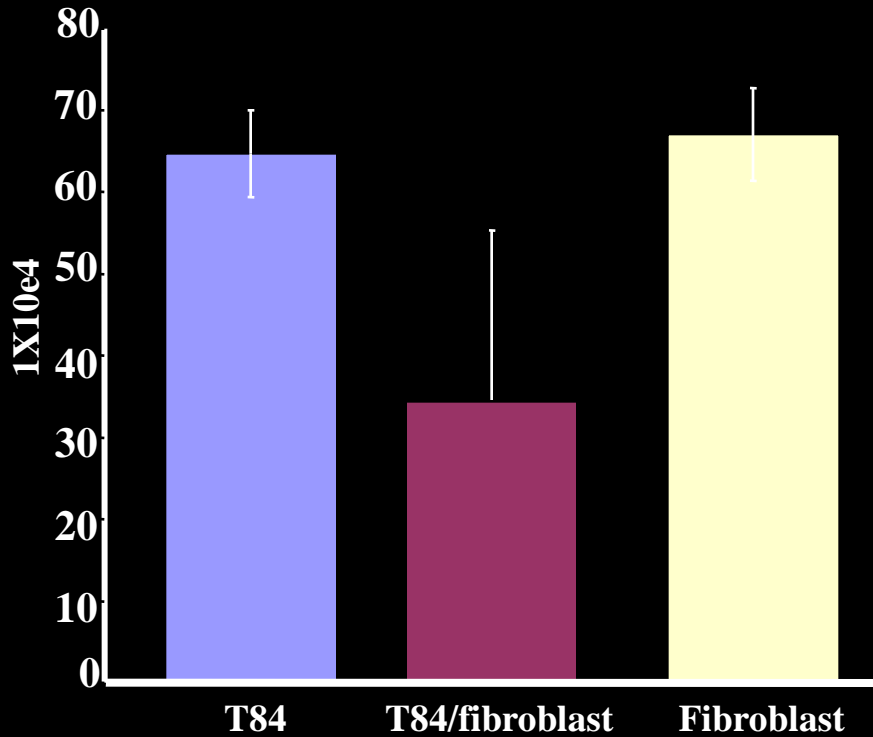
T84 cells	T84-CCD18 cells	CCD18 cells
214	1234	62
710	1483	58
576	1238	58
438	1480	57
<u>635</u>	<u>1779</u>	<u>52</u>
654	1598	55

The average for T84 cells = 654; average for T84/CCD18=1598; average for CCD18=55

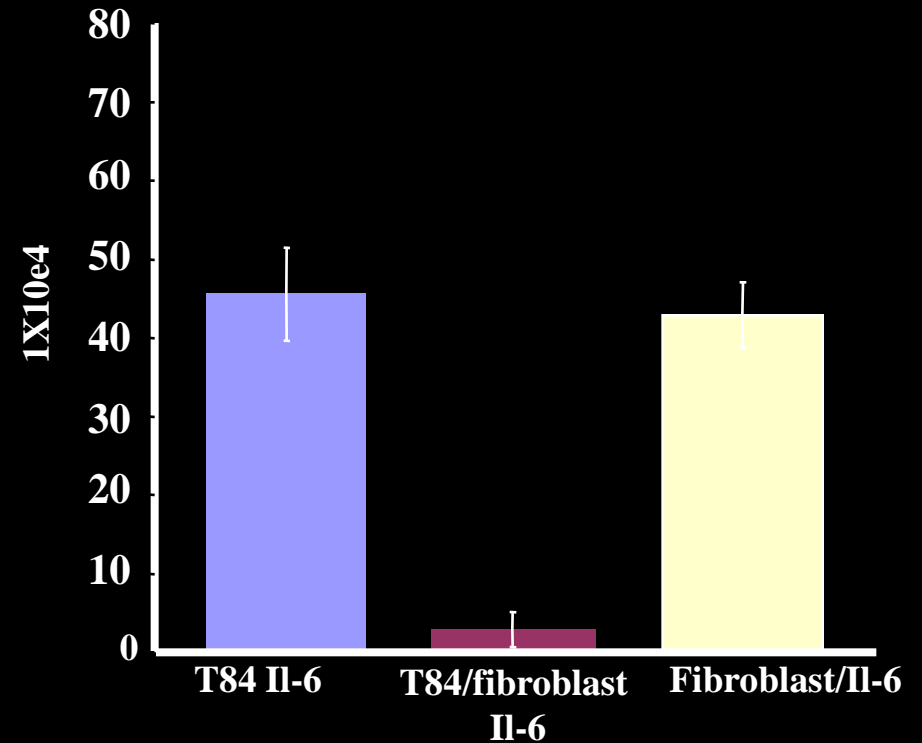
Previous reports: Co-Cultured fibroblasts and epithelial cells result in increase transepithelial resistance

PMN Transepithelial Migration across Intestinal Fibroblasts and Epithelial Cells

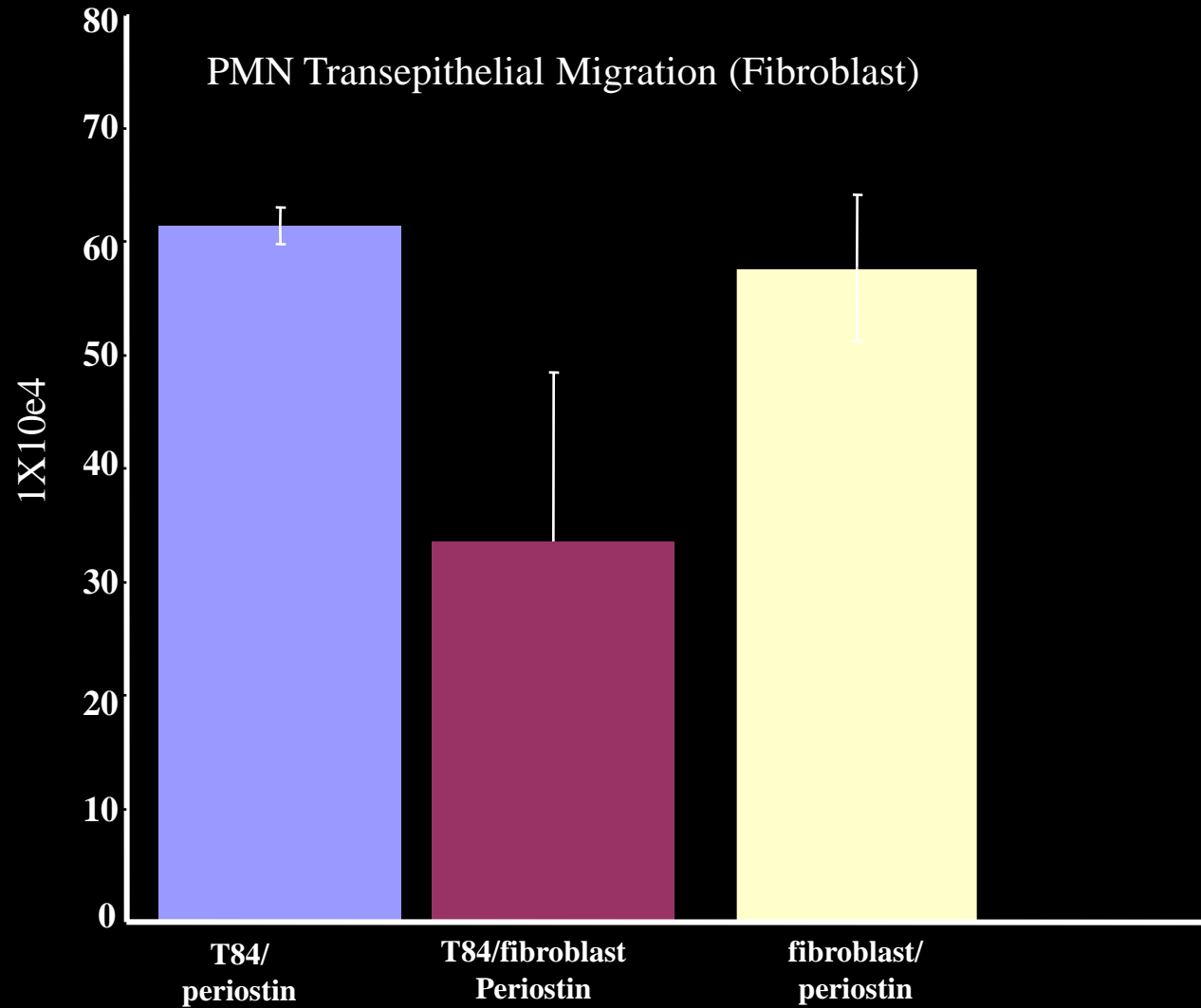
Transepithelial Migration



Transepithelial Migration Interleukin-6



Periostin eliminates the inhibition of transmigration following cellular exposure to IL-6



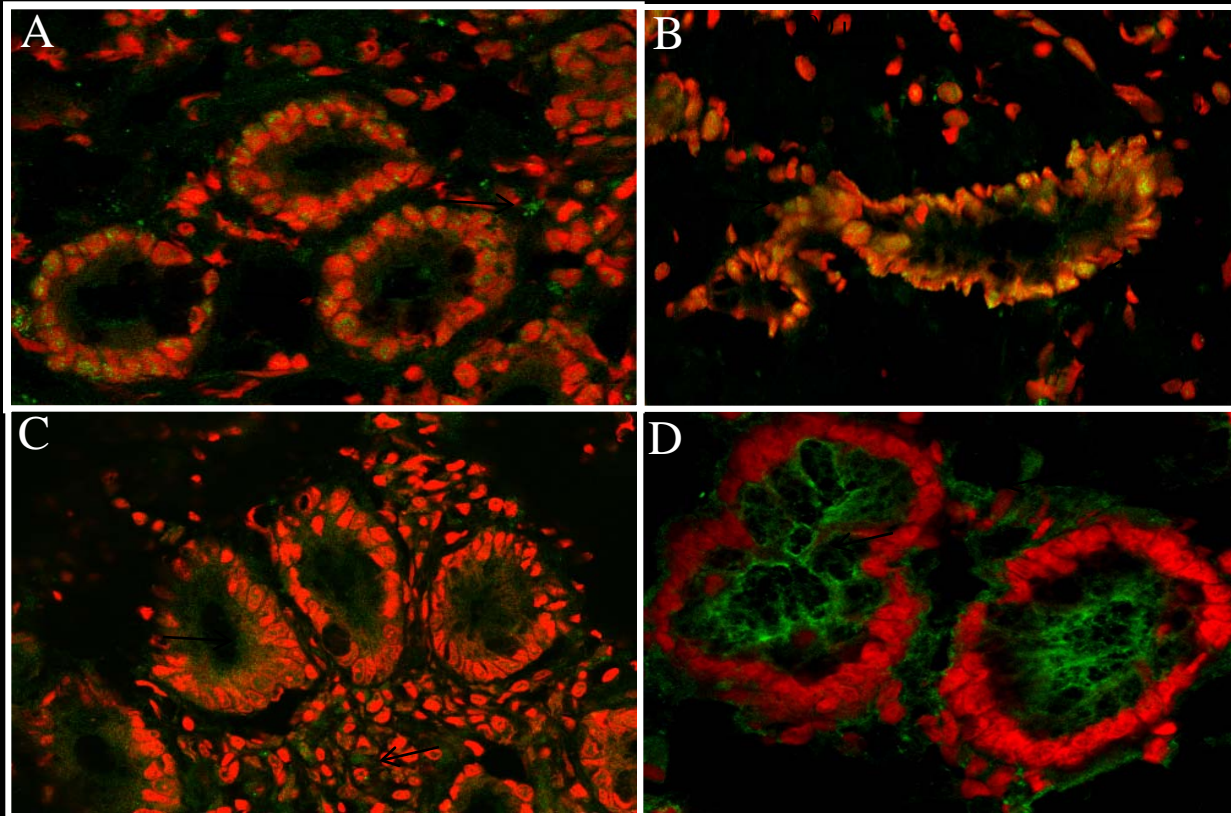
Periostin is localized in the human Intestine under normal conditions and under conditions of Inflammation

■ antigen (periostin)

■ nuclei

Normal

Cancer



Crohn's Disease

Ulcerative Colitis

Conclusions

PMN will migrate across intestinal fibroblasts and epithelial cells toward fMLP (formylated bacterial peptides)

IL-6 stimulation of fibroblasts combined with epithelial (T84) cells have an inhibitory effect on PMN transmigration

The introduction of purified periostin eliminates the inhibition observed with IL-6 stimulation of epithelial (T84) cells in combination with intestinal fibroblasts

Periostin is localized at the basal and apical poles of the crypt epithelium of both normal, cancer and IBD (Crohn's Disease and Ulcerative Colitis) intestines

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