

***Clostridium perfringens* as a Novel Therapeutic Vehicle in Inflammatory Bowel Disease**

Scott Plevy^{1,2}, Fengling Li^{1,2}, Yue Chen³, Shaival Dave^{1,2}, Jeremy Tilstra^{1,2}, Paul Robbins⁴, Bruce McClane⁴, Albert Baldwin⁵, Phalguni Gupta³

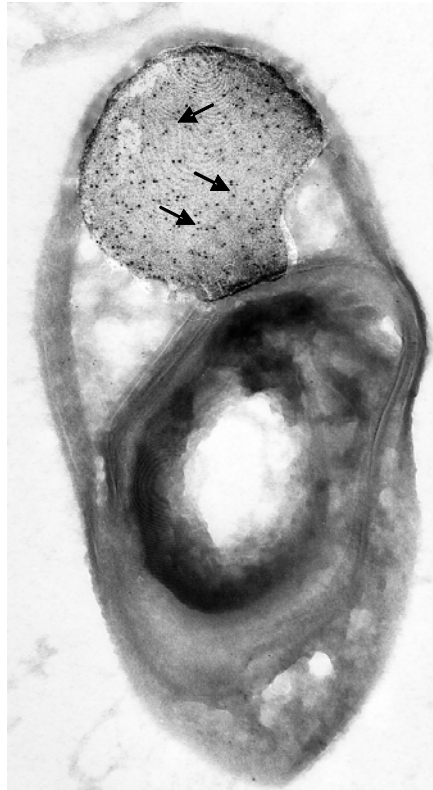
¹Department of Medicine; ²Department of Immunology;
³Department of Infectious Diseases and Microbiology, Graduate School of Public Health; ⁴Department of Molecular Genetics and Biochemistry; University of Pittsburgh School of Medicine, Pittsburgh, PA, U.S.A. ⁵Theralogics, Inc. Chapel Hill, NC, U.S.A.

Background

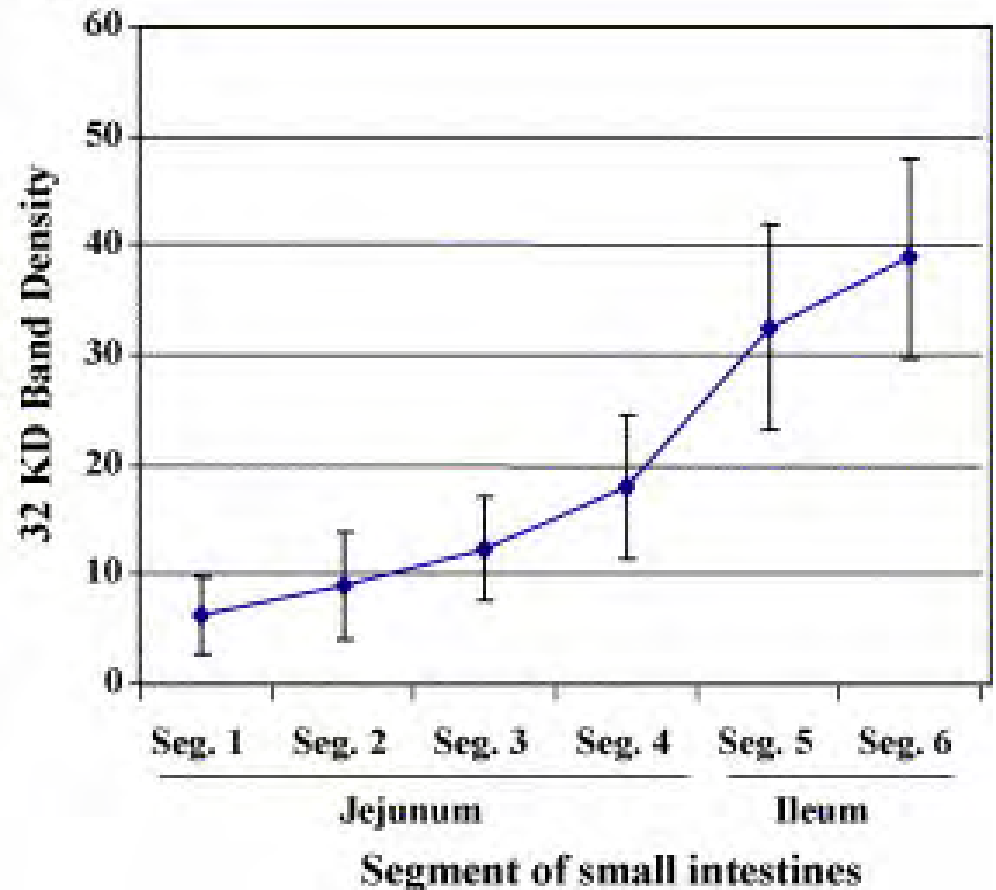
- ***Clostridium perfringens*** is an anaerobic, gram-positive, rod-shaped, encapsulated nonmotile bacterium.
- ***C. perfringens*** are present throughout the natural environment, including soil, foods, dust, and importantly, the intestinal tract of humans and domestic animals.
- A non-cytotoxic (plasmid-based cytopathic gene deleted) CP provides an attractive vehicle to deliver therapeutic proteins to the mucosal surface of the small intestine, especially the terminal ileum.
- This proposal will develop and validate a genetically altered strain of CP that expresses murine IL-10 during sporulation.

SIV p27 in inclusion body of endospore-containing *C. perfringens* is delivered to the murine ileum

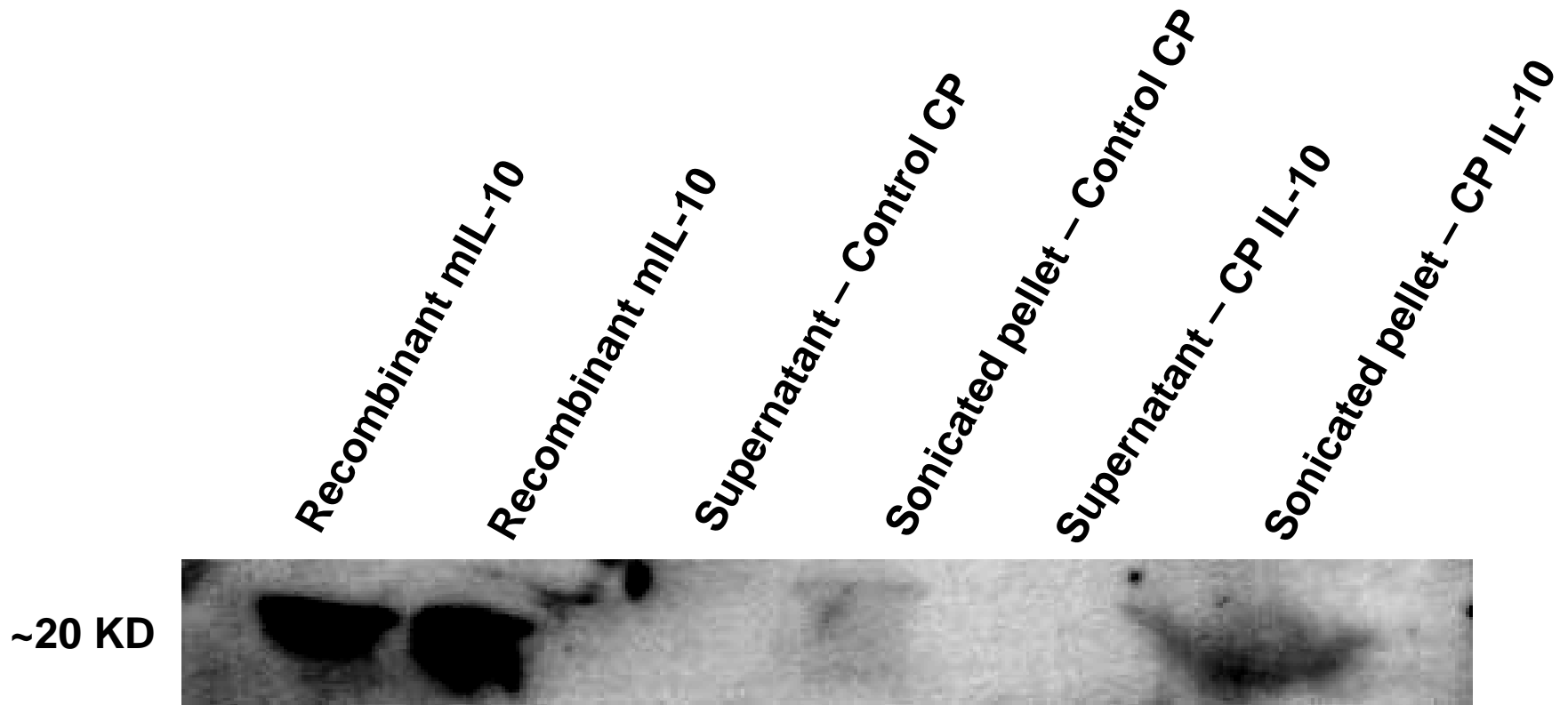
A



B



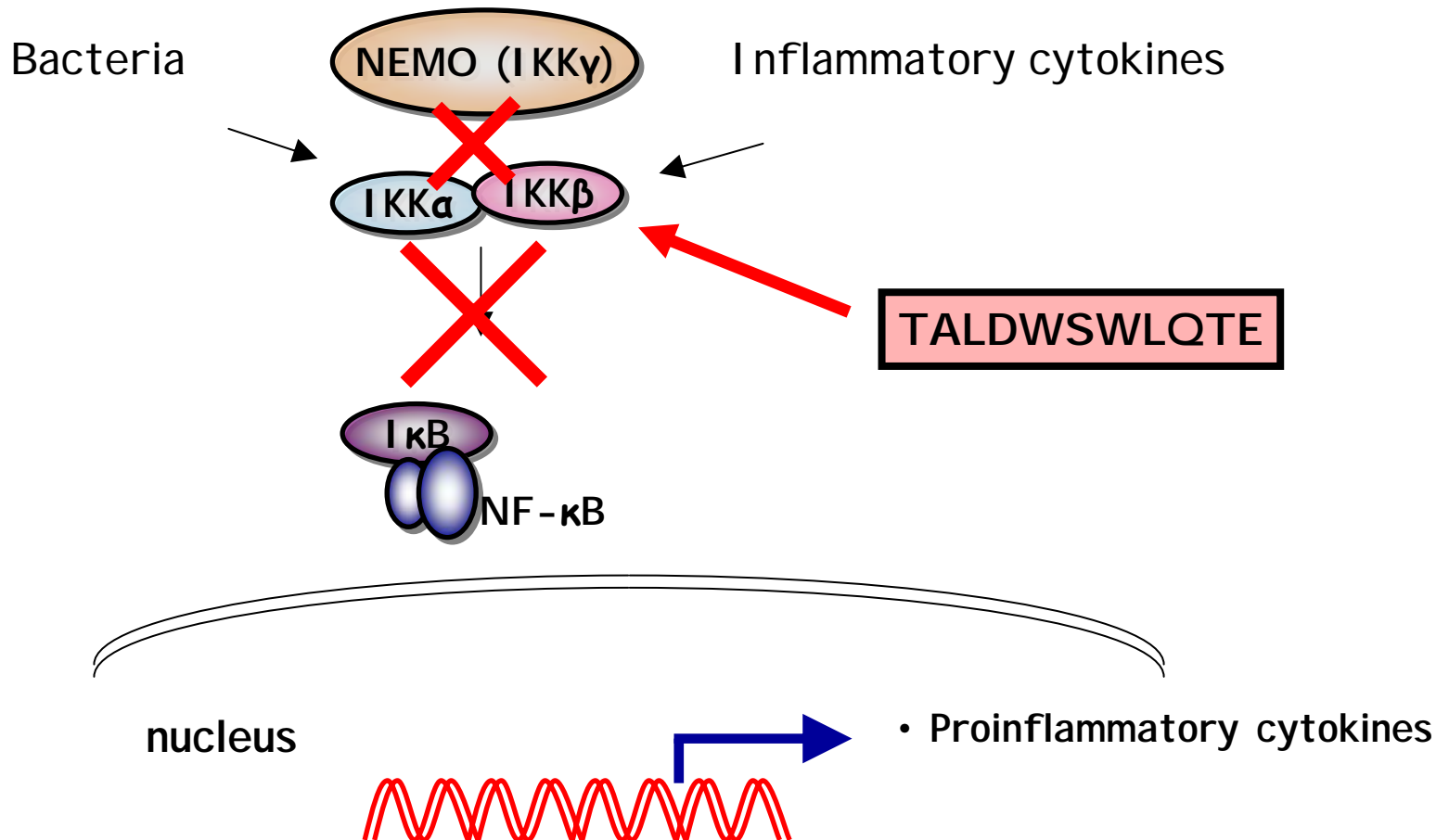
Sporulating *C. perfringens* cultures express IL-10 from the *cpe* promoter



Pitfalls

- **IL-10 immunoreactivity detected by ELISA/Western, but at low concentrations.**
 - Ineffective concentrations to demonstrate IL-10 bioactivity (cytokine inhibition assay).
 - Most IL-10 found as insoluble precipitate in inclusion bodies of sporulating *C. perfringens*.
 - Not effective in vivo in IL-10^{-/-} mice.
- **Search for small immunologically active peptides that may be expressed in *C. perfringens* without solubility problems.**

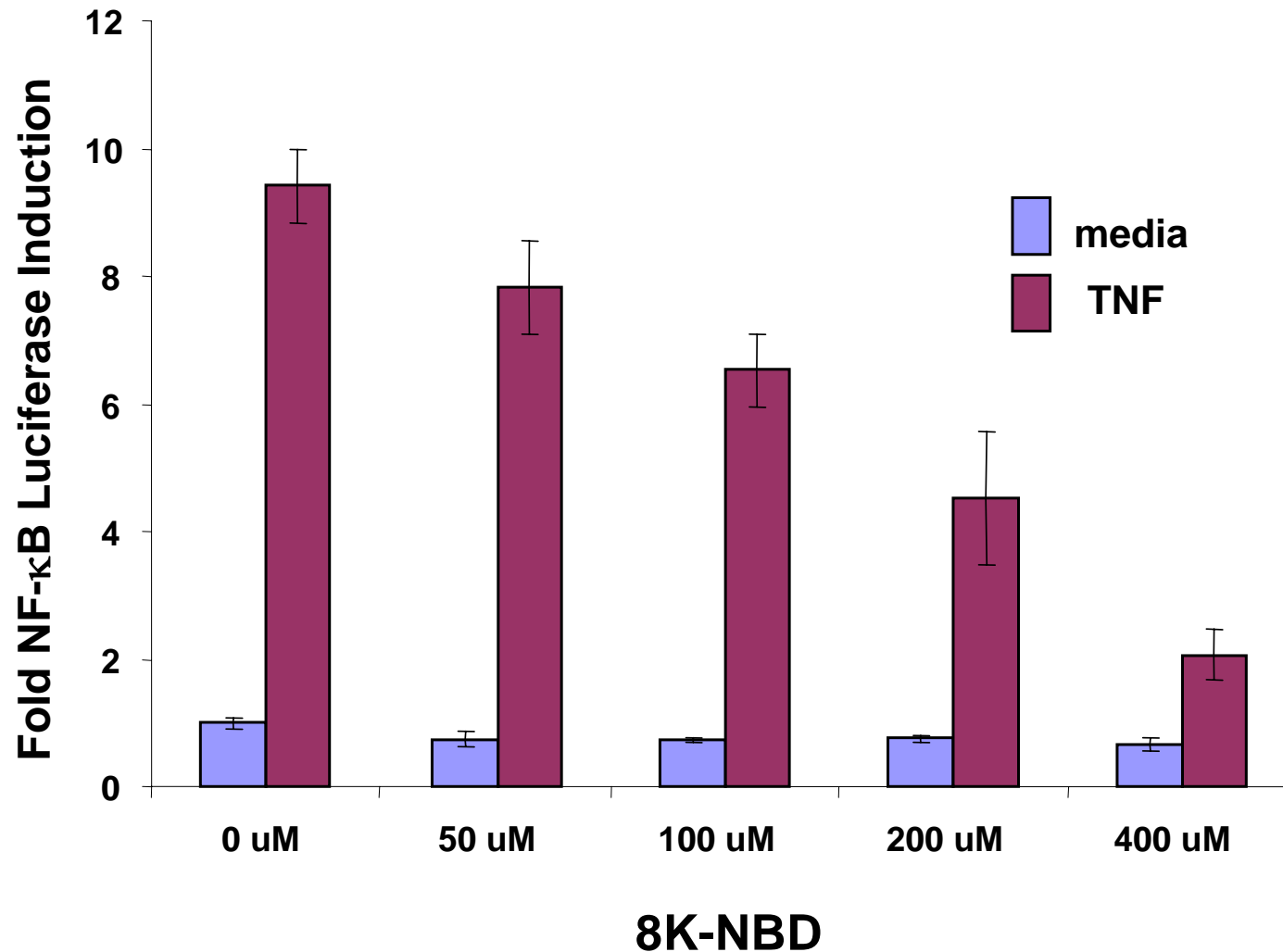
NEMO binding domain (NBD) peptide disrupts association of NEMO with IKKs



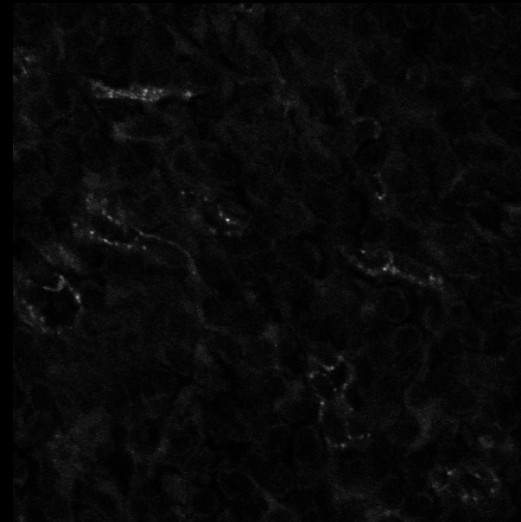
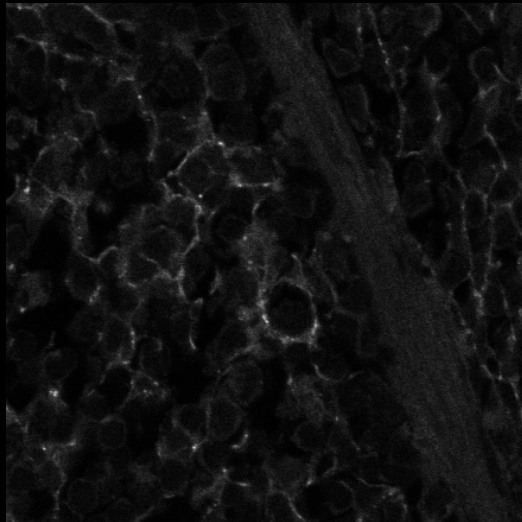
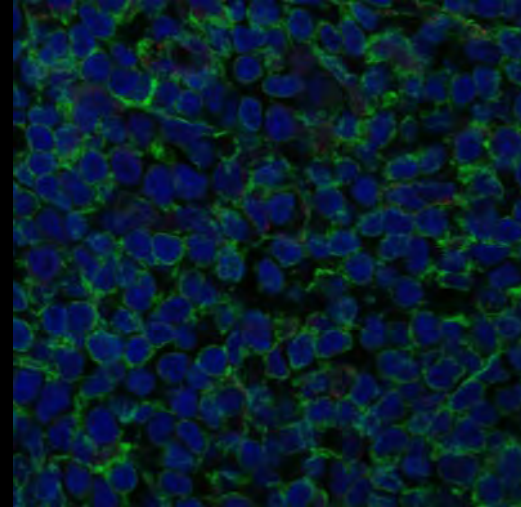
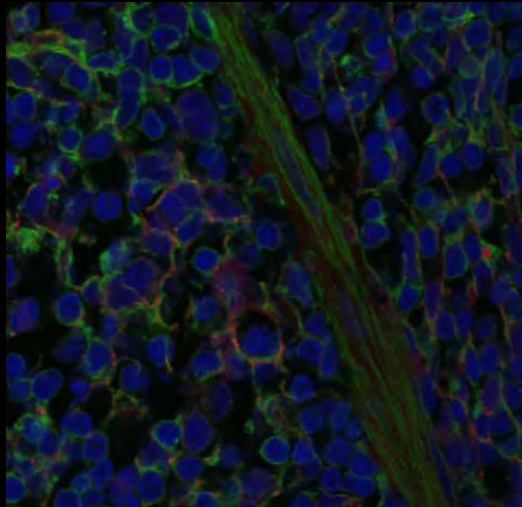
Introducing NBD via PTDs

- **Introduced into cells via protein transduction domains (PTDs).**
 - PTD: HIV-TAT, antennapedia, and 8K
- ***In vivo* treatment with PTD-NBD in murine collagen induced arthritis and experimental allergic encephalomyelitis demonstrated:**
 - **attenuated disease activity**
 - **decreased pro-inflammatory cytokine expression**

8K-NBD dose dependently inhibits NF- κ B activation in cells



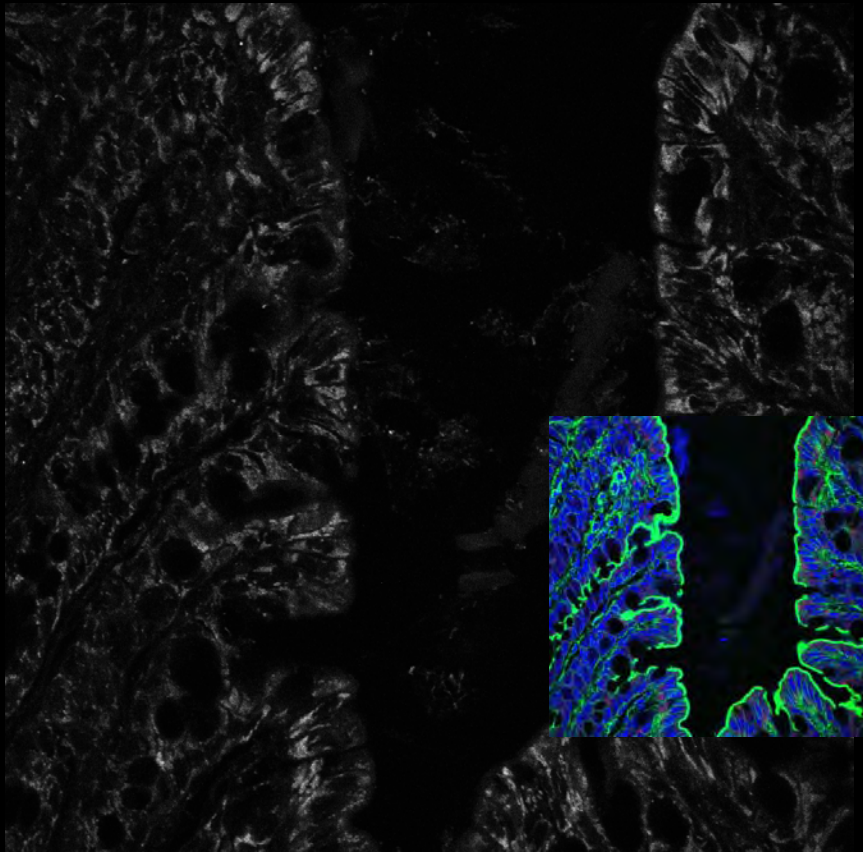
IP 8K PTD detected in splenocytes



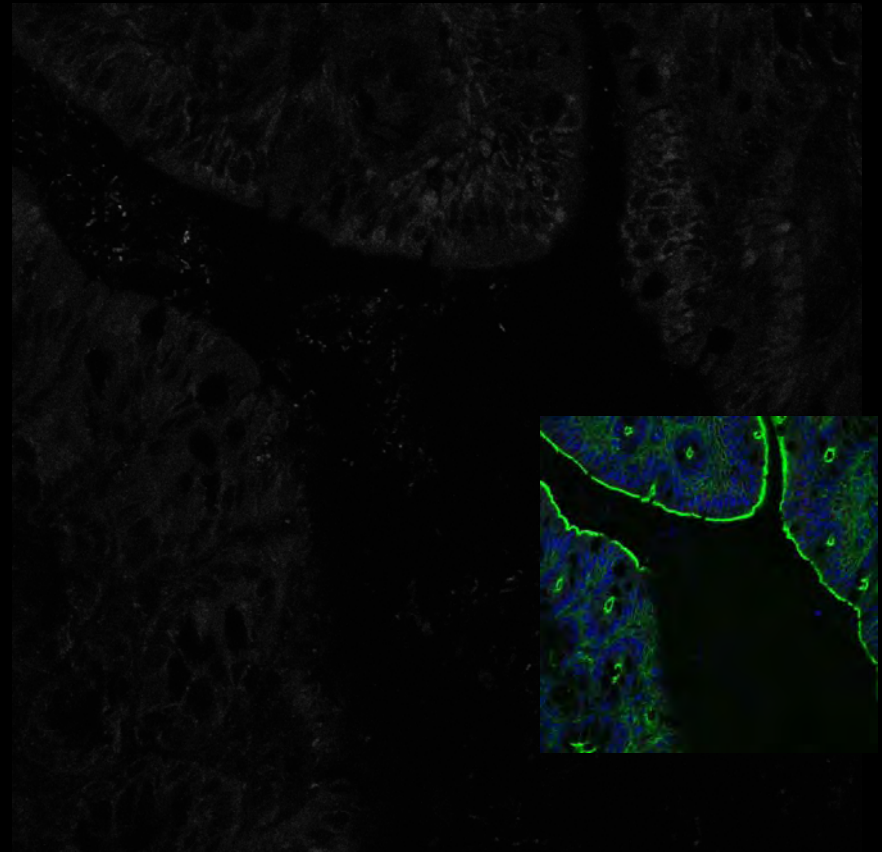
8K-biotin- streptavidin cy3

RAN-biotin- streptavidin cy3

Intrarectal 8K PTD

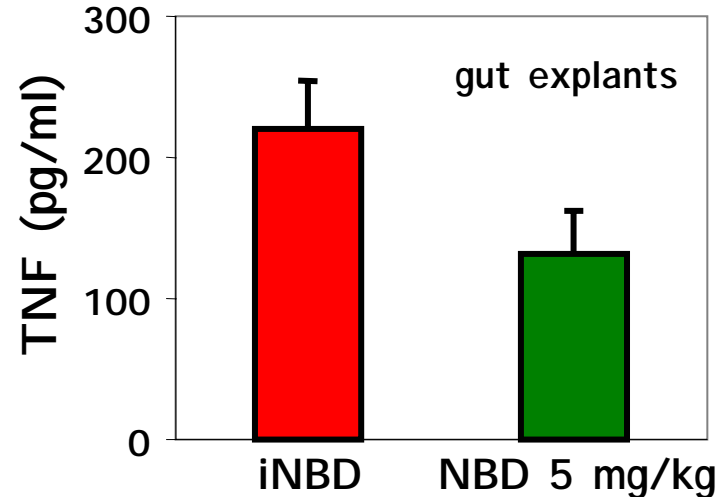
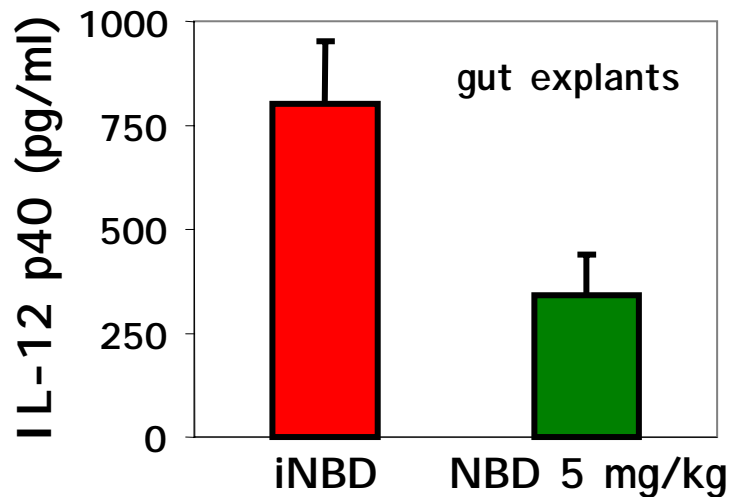
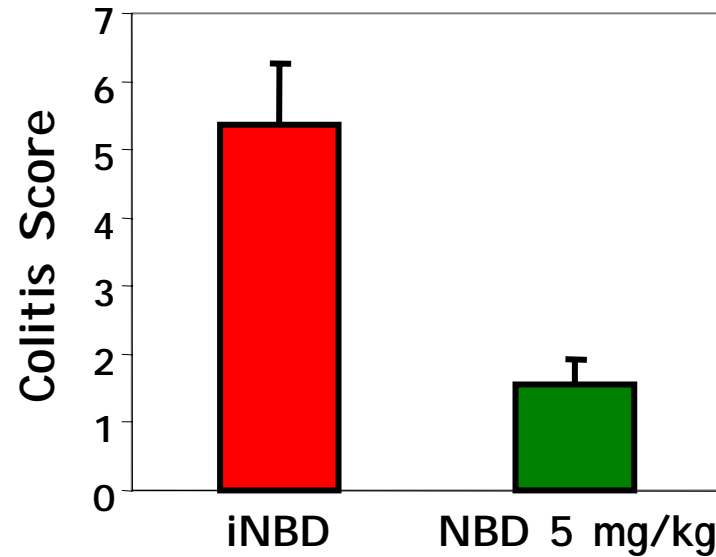


8K-biotin- strepavidin cy3



RAN-biotin- strepavidin cy3

IP administration of 8K-NBD to IL-10^{-/-} mice for 2 weeks ameliorates colitis.



Conclusions

- **PTDs transduce cells *in vitro***
- **PTDs transduce cells *in vivo* when given systemically and locally**
- **8K PTDs have greater transduction potential than TAT *in vivo*.**
- **IP administration of 8K-NBD ameliorates colitis in IL-10^{-/-} mice.**

Future Goals

- **Comparison of efficacy of PTD-NBD administered oral, intrarectal, and systemically in mouse models of IBD.**
 - Define target cells, MOA, toxicity
 - Collaboration with Theralogics, Inc./UNC-Chapel Hill to develop as a therapeutic in IBD (NIH/NIDDK 1 R41 DK074193-01).
- **Test *C. perfringens* expressing PTD-NBD in mouse models of IBD.**
- **Use of nonpathogenic *Clostridium* strains, α toxin deleted *C. perfringens*, chromosomally based cpe promoter constructs as delivery vehicles.**