

# **Downregulation of TFFs expression in gastrointestinal cell lines by cytokines and nuclear factors**

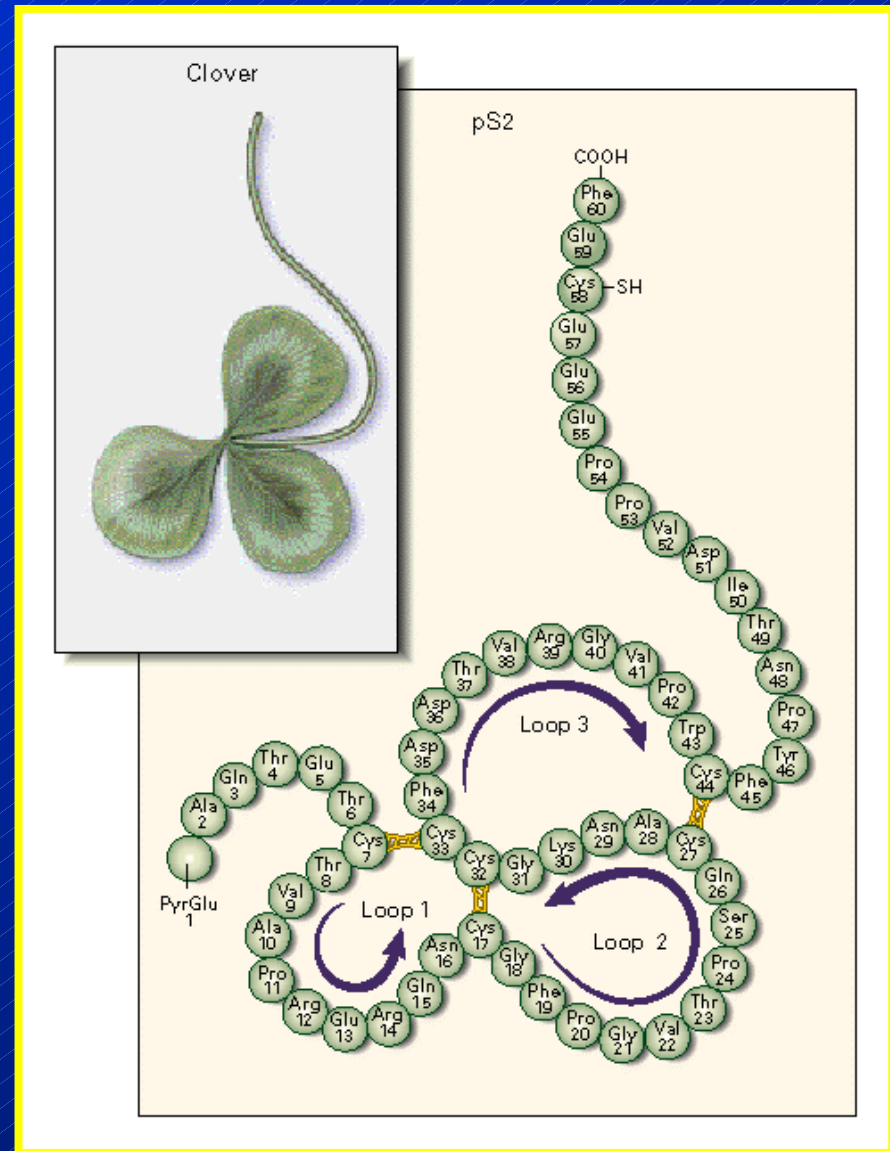
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# MAINTENANCE OF MUCOSAL INTEGRITY OF GASTROINTESTINAL TRACT

- Absorptive surface of GI tract  $> 10 \text{ m}^2$
- Depletion of  $\sim 200 \text{ g}$  of epithelial cells/day

# TFFs (Trefoil Factors)

- group of small proteins with 1 or 2 characteristic 3-leaved structure (“trefoil”-domain) secured by disulphide bonds
- mucin producing cells of gastrointestinal and respiratory tract, salivary glands, uterus, conjunctiva
- antiapoptotic and motogenic activity  $\Rightarrow$  cell migration in process of restitution
- maintaining of surface integrity





Playford *et al.* (1996):

transgenic mice overexpressing human TFF1

☉ *increased resistance to intestinal damage*

Mashimo *et al.* (1996): mice lacking Tff3 protein

• impaired mucosal healing and poor epithelial regeneration

• mice died from extensive colitis after introducing colonic injury

☉ participates in the *healing & repairing mechanisms* in the intestinal mucosa

Lefebvre *et al.* (1996): mice lacking Tff1 protein

• adenomas and in 30% of the homozygous animals, multifocal intraepithelial and intramucosal carcinomas

☉ may function as a *gastric-specific tumour-suppressor gene*

Farrel *et al.* (2002): mice lacking TFF2 protein

• increased degree of gastric ulceration after indomethacin administration

## Inflammatory Bowel Disease (IBD):

- Ulcerative colitis and Crohn's disease
- characterised by chronic inflammatory processes
- Inflamed intestine of IBD patients have increased amounts of proinflammatory cytokines: IL 1 $\beta$ , IL 6 and TNF $\alpha$
- IL 1 $\beta$  and TNF $\alpha$  act through NF $\kappa$ B transcription factors  
IL 6 acts through CCAAT/enhancer binding protein (C/EBP $\beta$ )
- TFFs as intestinal „protective“ peptides could play role in development of IBD

↓ ???

Effect of IL 1 $\beta$ , IL 6 and TNF $\alpha$  and  
NF $\kappa$ B and C/EBP $\beta$  on TFFs expression

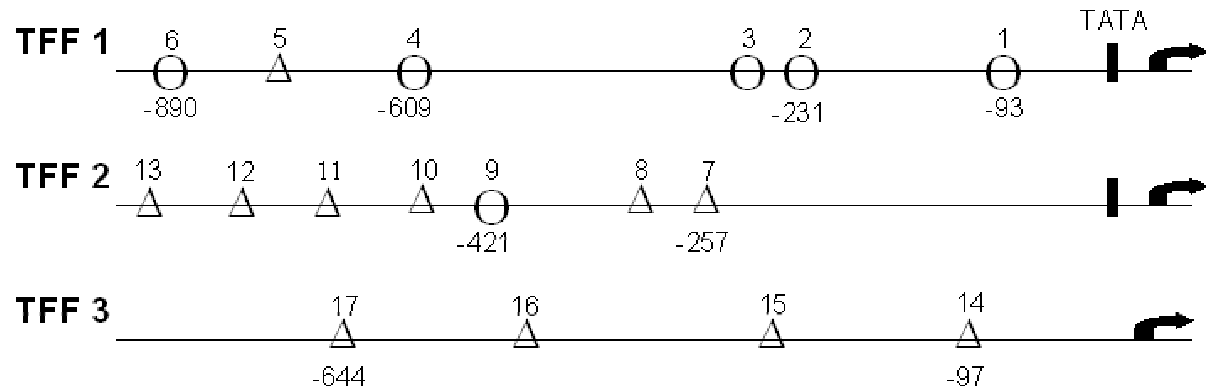
# Identity with the consensus binding site

## NFκB

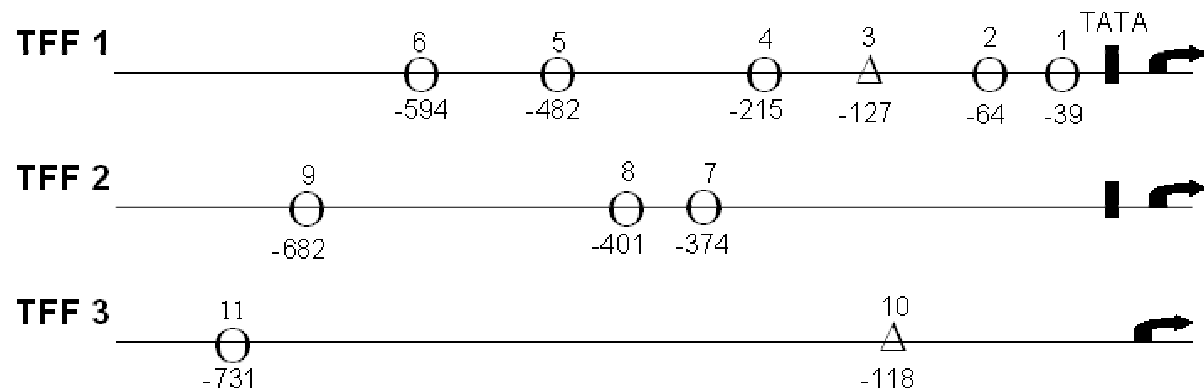
(O) 90 %

(Δ) 80 %

**A**



**B**



## C/EBPβ

(O) 100 %

(Δ) 85 %

# METHODS

colon adenocarcinoma cell line HT 29

A) cells were transfected with TFF1, TFF2 or TFF3 reporter gene vectors  
(Dual Luciferase reporter assay, Promega)

Monitoring the effects on TFFs reporter gene after:

- 1) stimulation by IL 1 $\beta$ , IL 6 or TNF $\alpha$
- 2) cotransfection of NF $\kappa$ B ( p50/p65) and/or C/EBP $\beta$  expression vectors

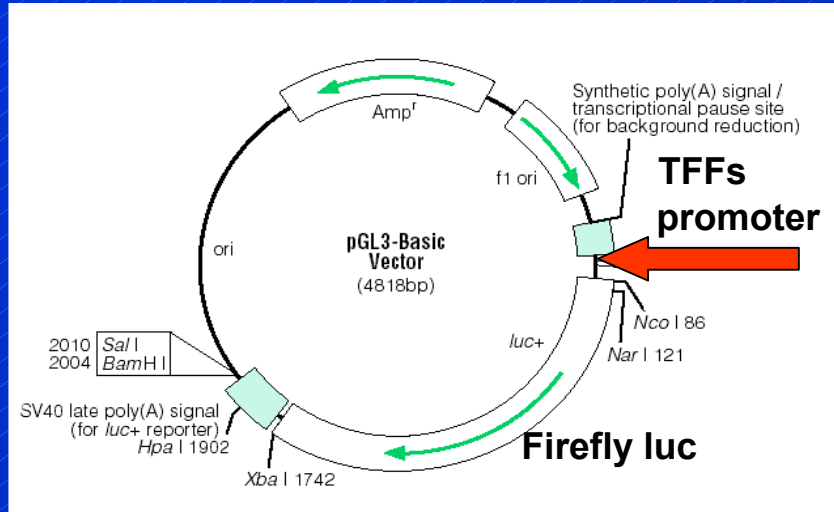
B) Monitoring the expression of the endogenous trefoil genes in HT 29  
by qPCR after stimulation with TNF $\alpha$ , IL 1 $\beta$  and/or IL 6

detection SYBRGreen ; i-Cycler iQ (Bio-Rad)  
expression normalized to GAPDH

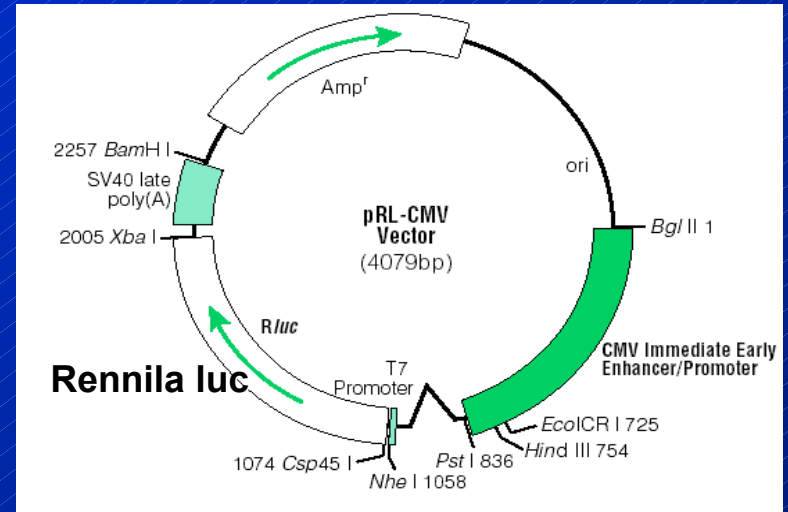
All experiments repeated minimal 4 times (in triplicates)  
and analyzed by one way ANOVA

# Dual Luciferase reporter assay

## Experimental reporter vector



## Control reporter vector



Transient transfection (HT-29)



6h

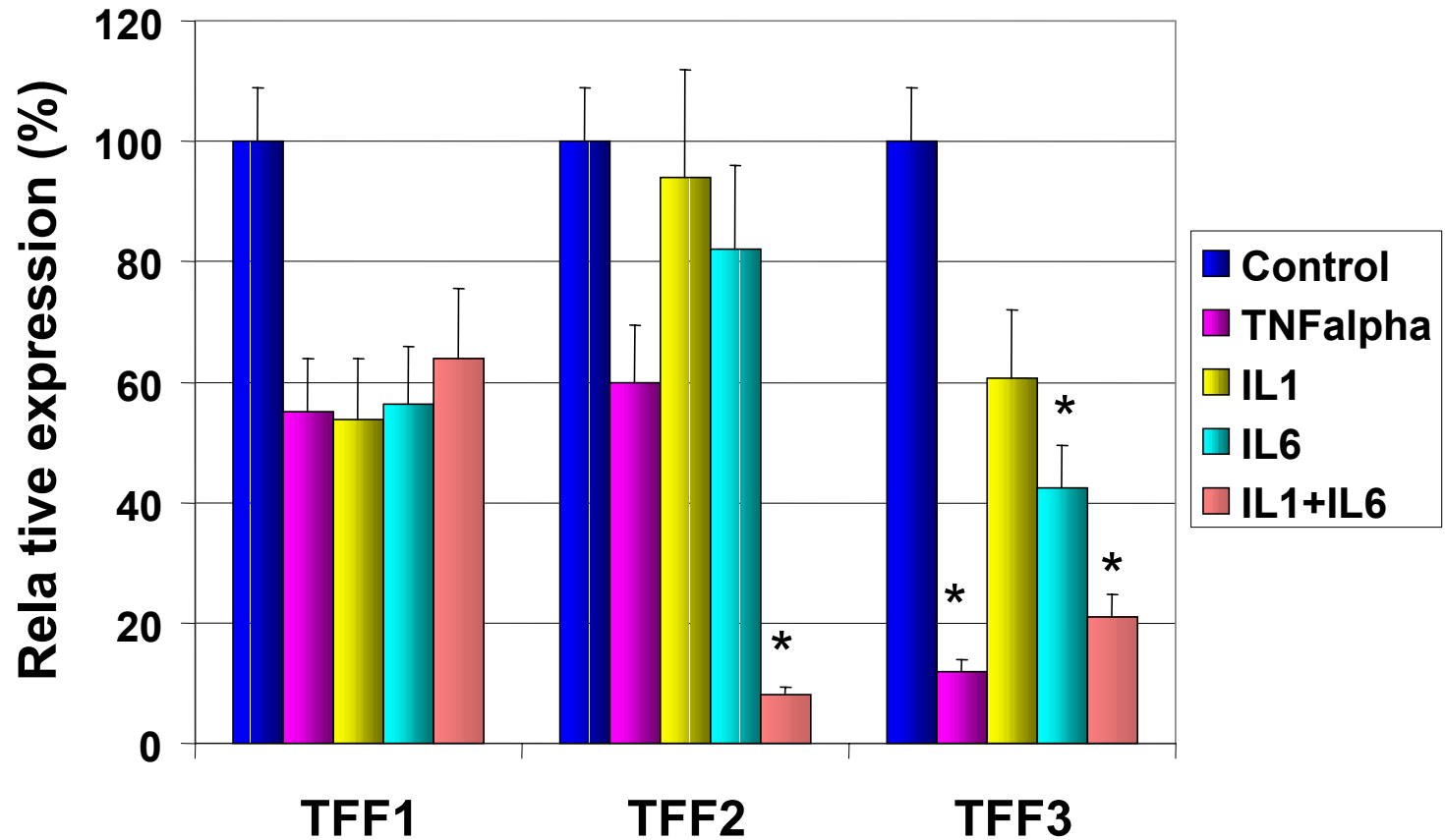
medium exchange and stimulation (IL 1 $\beta$ , IL 6 or TNF $\alpha$ )



42h

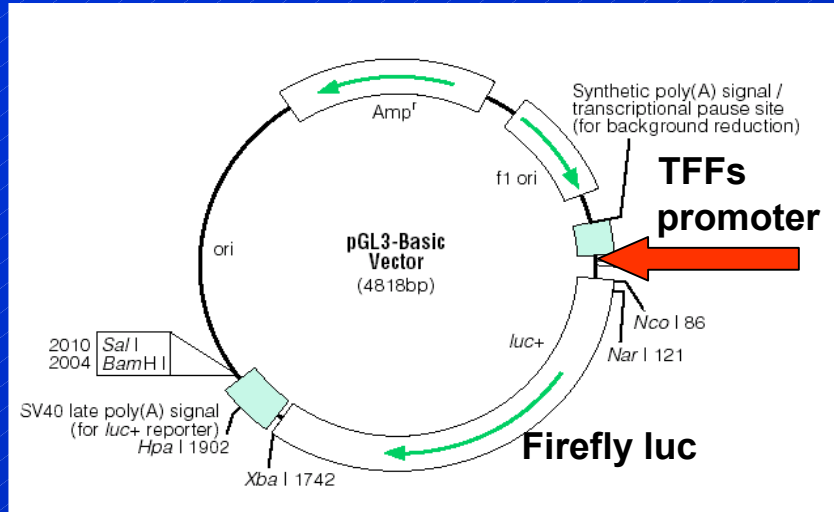
Light Intensity Firefly/Renilla Luciferase vs. unstimulated control

# Reporter gene assay

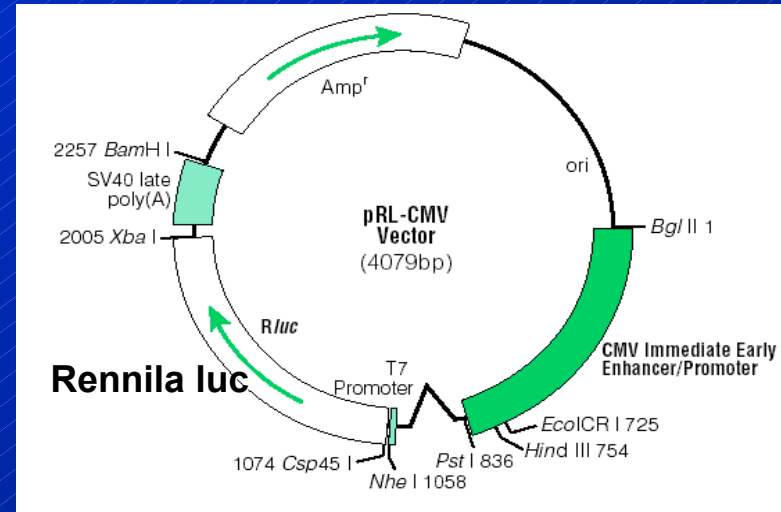


# Dual Luciferase reporter assay

## Experimental reporter vector



## Control reporter vector



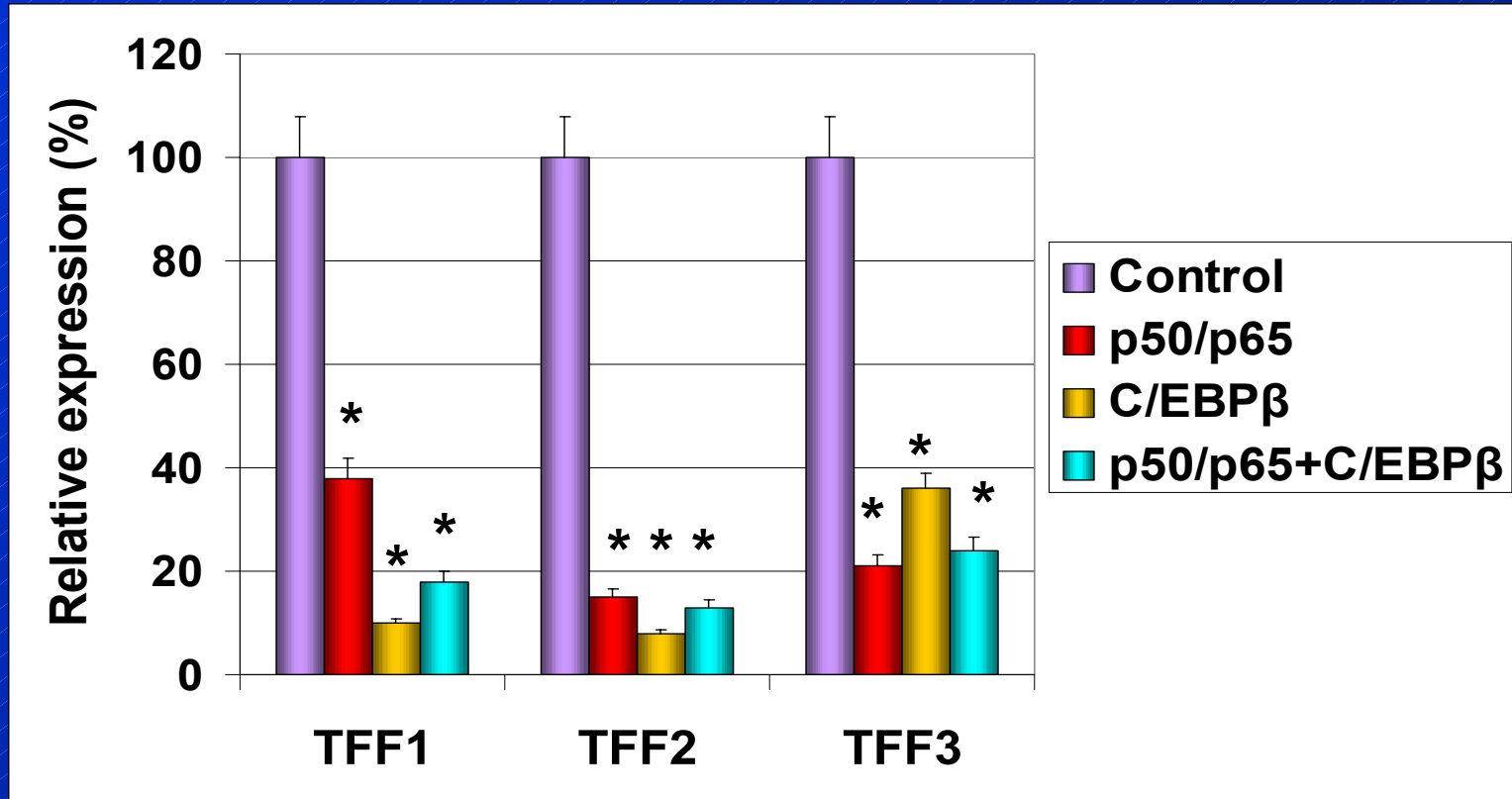
+

plasmid constitutively  
expressing NF $\kappa$ B and C/EBP $\beta$

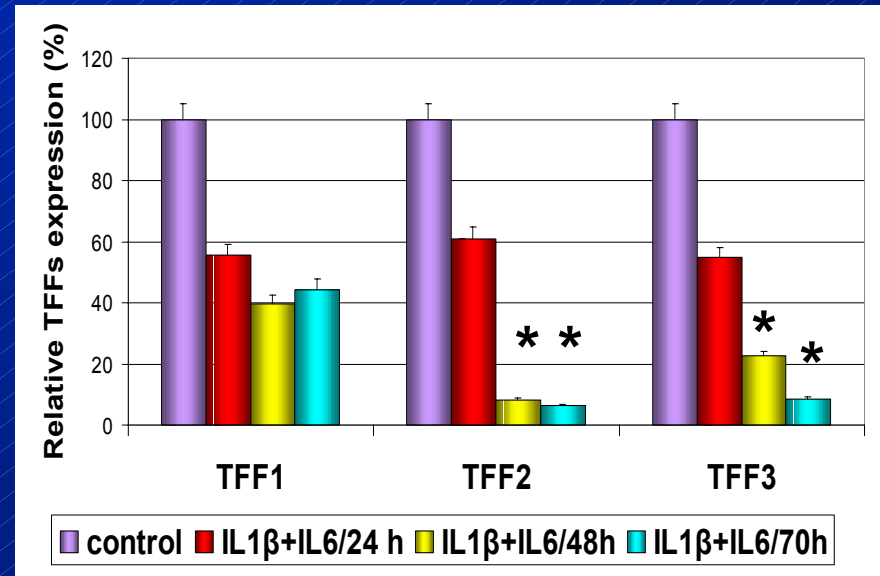
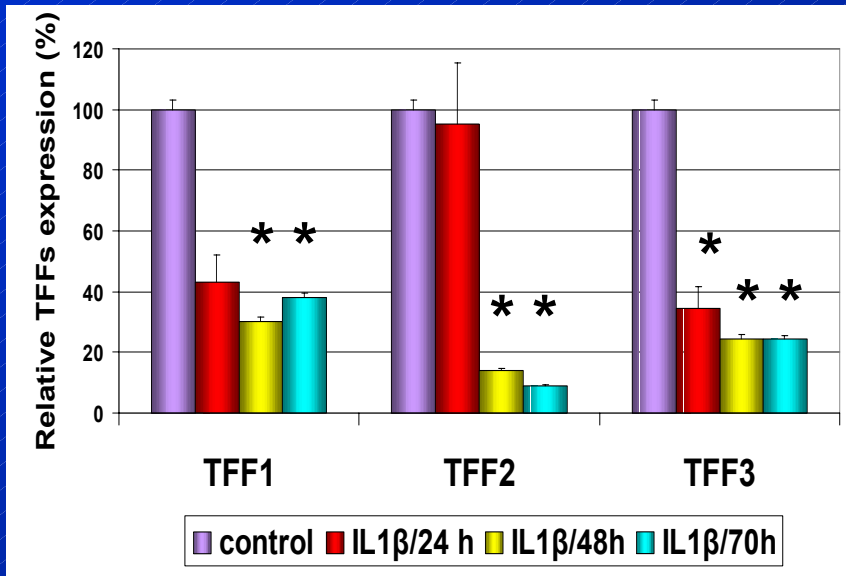
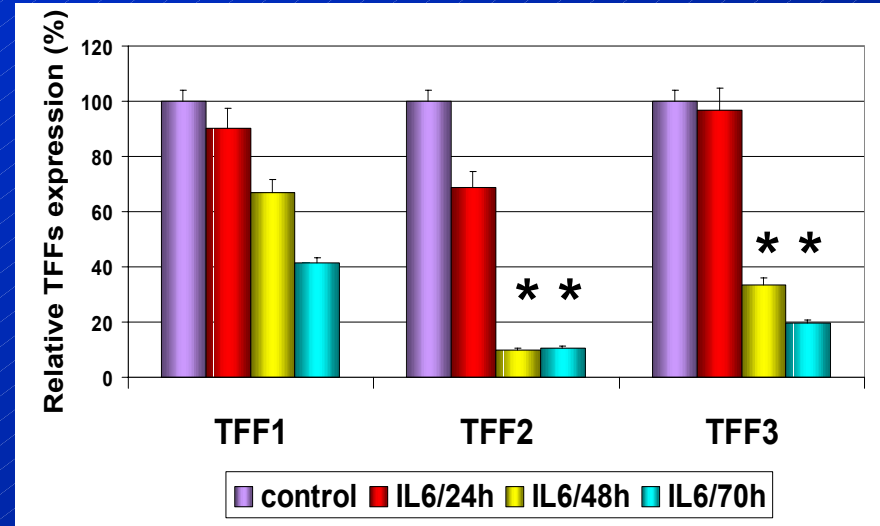
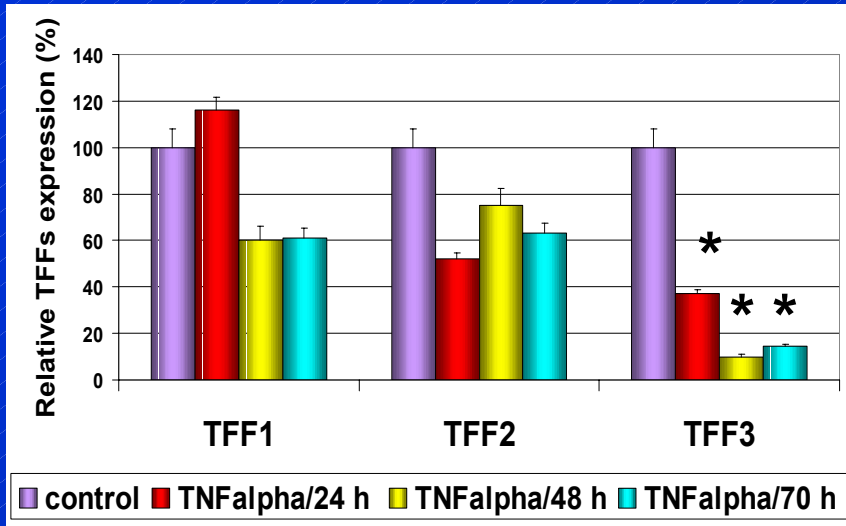
⇓ 48h

Light intensity Firefly/Renilla Luciferase vs. unstimulated  
control

# Downregulation of TFF reporter gene constructs by NF $\kappa$ B and C/EBP $\beta$



# Quantitative Real Time PCR results



# Conclusion

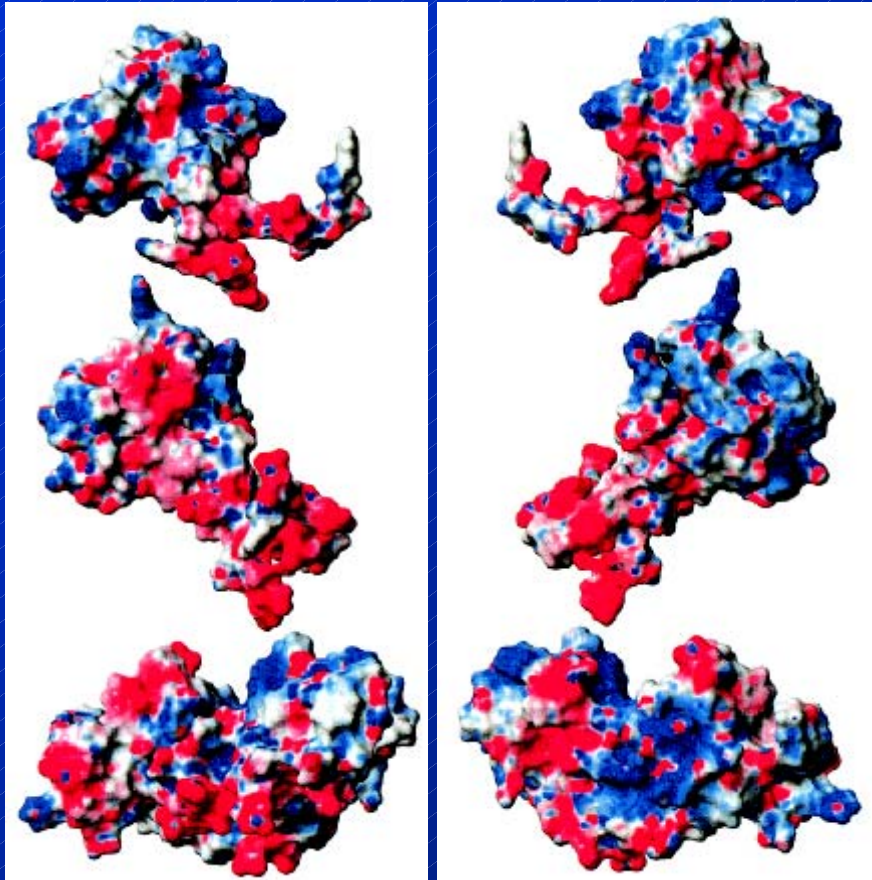
- proinflammatory cytokines IL 1 $\beta$ , TNF $\alpha$  and IL 6 downregulate expression of TFFs (3-11 fold reduction measured by qPCR)
- TFF3 downregulation through NF $\kappa$ B and C/EBP $\beta$  may reflect the situation *in vivo* and contribute to ulceration during IBD

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# Comparison of molecular surfaces of hTFFs

TFF3



TFF1

TFF2

Rotated for 180°



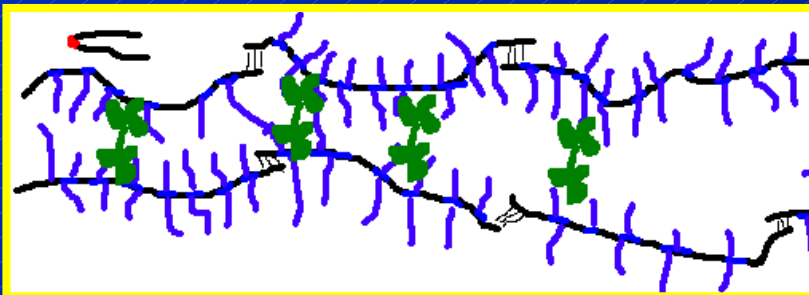
mucins

+



trefoil peptides

↓



*(Langer et al., 2001)*