



# Modulation of abdominal pain by probiotics

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## Functional gastrointestinal (GI) wellbeing

Up to 70% suffer from functional GI symptoms -  $\frac{3}{4}$  do not seek medical care

Chronic functional GI disturbances common – transient disturbances a rule

- Criteria exist for a large array of functional bowel disorders (FBDs)
  - Irritable bowel syndrome (IBS)
  - Functional bloating
  - Functional abdominal bloating
  - Functional constipation
  - Functional diarrhea
  - Functional abdominal pain syndrome



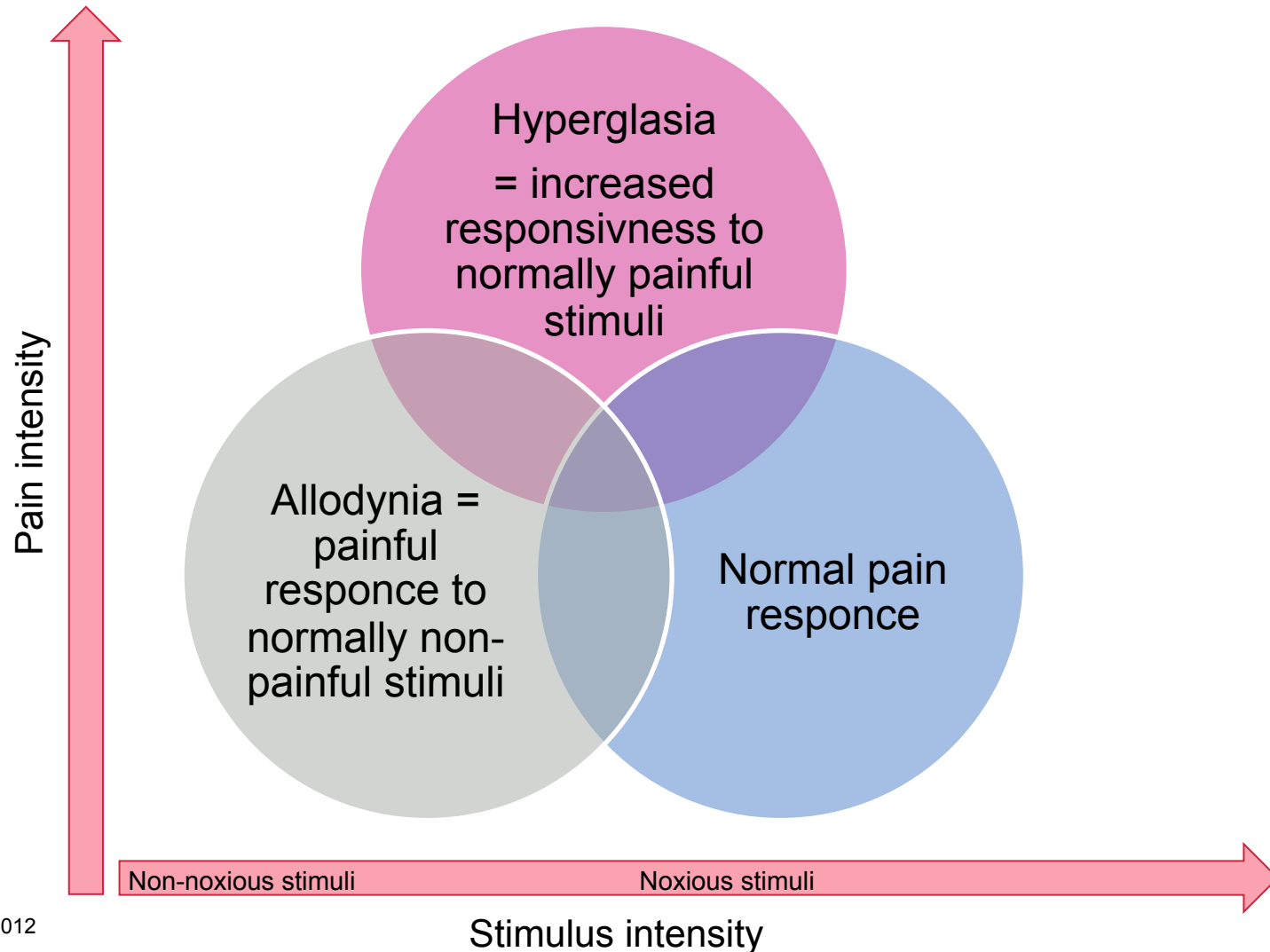
## Rome III criteria for IBS

(Longstreth et al., Gastroenterol 2006)

Recurrent abdominal **pain or discomfort** at least 3 days per month in the last 3 months associated with 2 or more of the following

- improvement with defecation
- onset associated with a change in frequency of stool
- onset associated with a change in form of stool

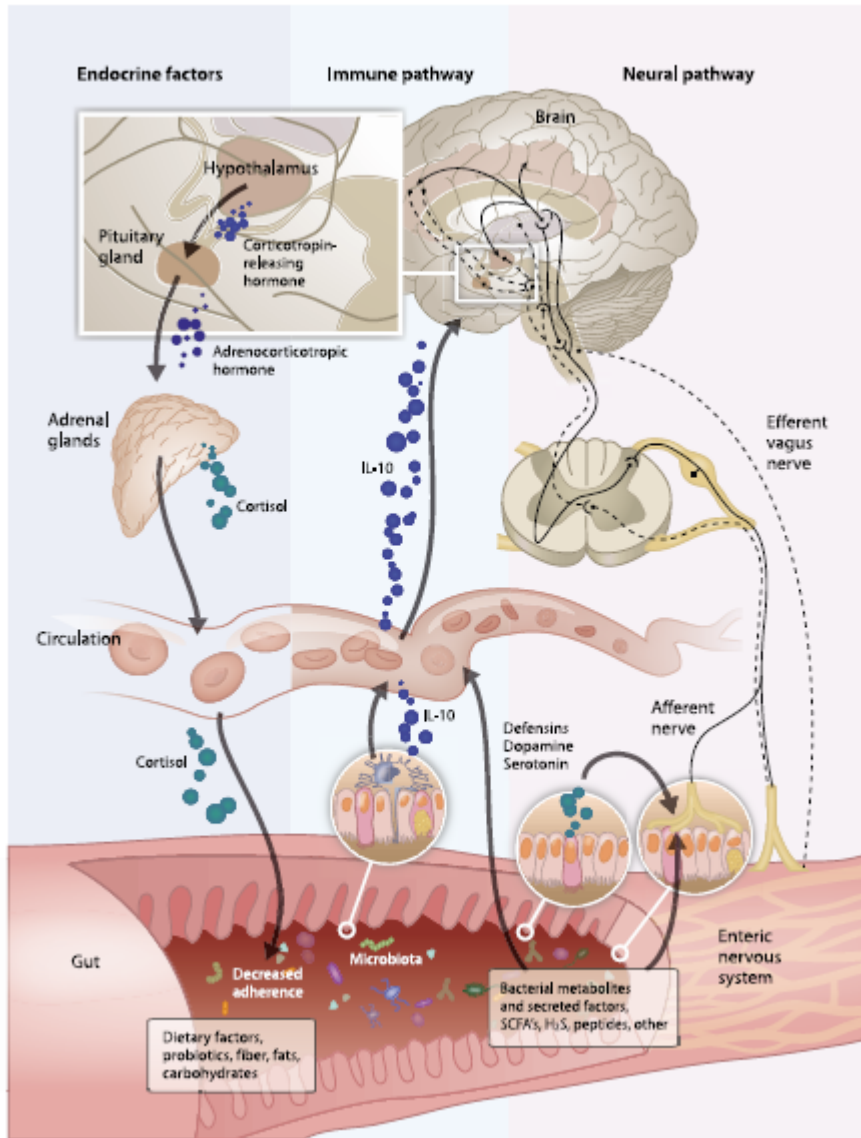
# Sensitization to pain



# **Abdominal pain is a common symptom attributed to visceral hypersensitivity**

**Experimental and clinical data suggest that changes in gut flora may be a basis for the variability of abdominal symptoms observed in functional gastrointestinal disorders and may be prevented by specific probiotic administration (1-4).**

1. Verdu EF et al. Gut 55, 182-90 (2006)
2. Kamiya T et al. Gut 55, 191-6 (2006)
3. Kajander K et al. Aliment. Pharmacol. Ther. 22, 387-94 (2005)
4. O'Mahony L et al. Gastroenterology 128, 541-551 (2005).



## Microbiome – gut – brain

- Bi-directional
  - Early life development essential for balanced function
  - Endocrine, immune, neural and intestinal factors
  - Stressors can disturb (psychological, infectious, etc.)
- (Chichlowski and Rudolph, JNM, 2015)

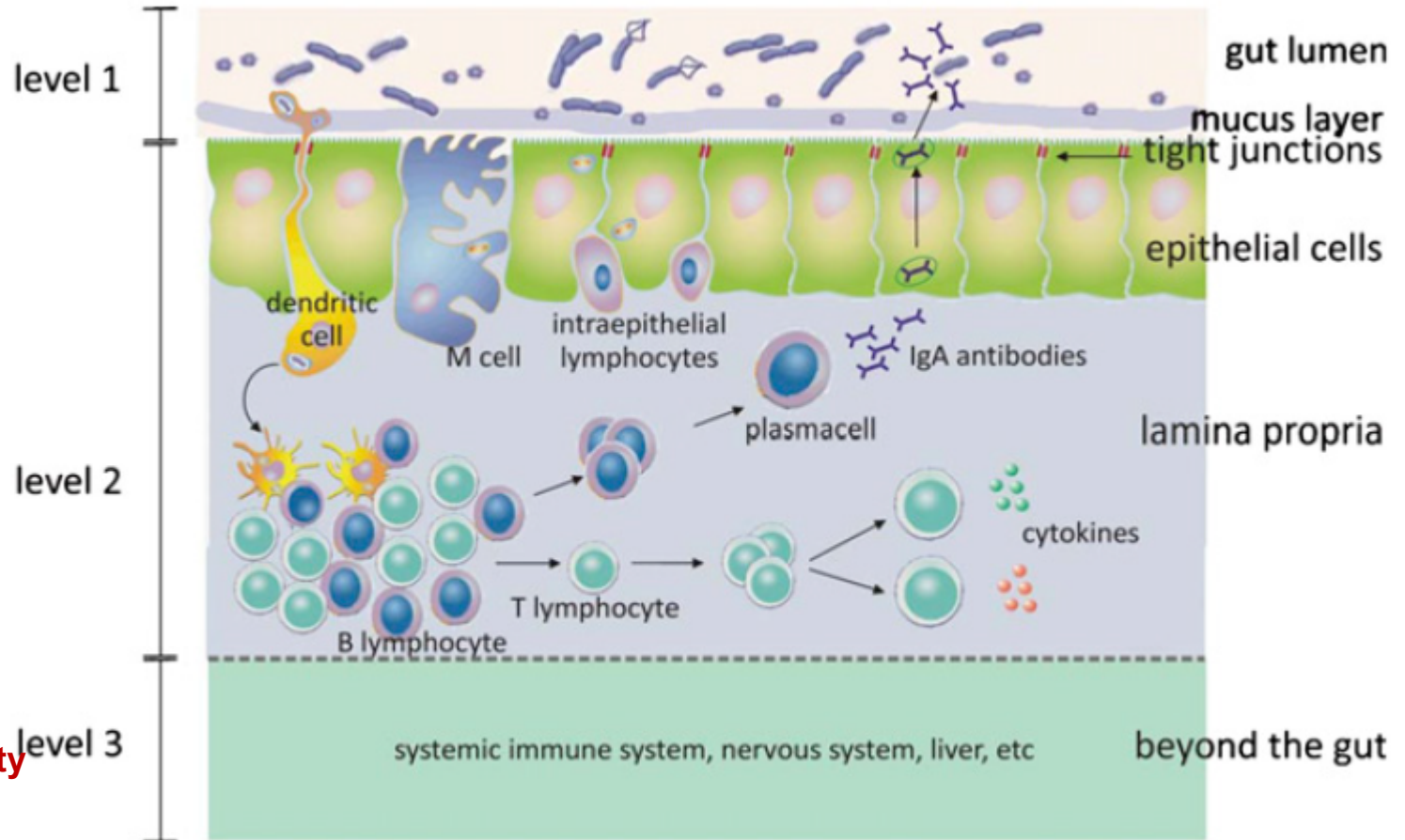
# Levels of action of probiotics

”Live microbes that, when administered in adequate amounts, confer a health benefit on the host” (FAO/WHO 2002; Hill et al., 2014)

**Recilience**  
**Composition**  
**Metabolites**

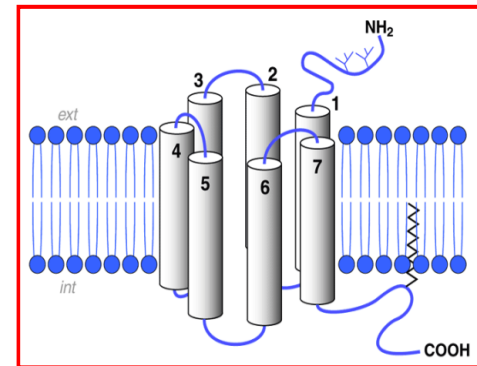
**Tight junction integrity**  
**Epithelial cell proliferation**  
**Immune effects**  
**Mucosal gene expression**

**Systemic immunity**  
**Host health**

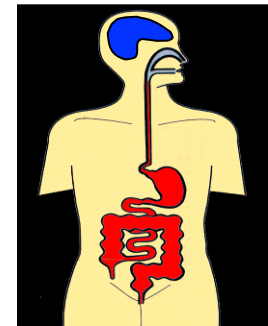


# Key regulators of pain

- 3 receptors (GPCR) are mainly involved in the regulation of pain \*:
  - Mu opioid receptor (MOR)
  - Cannabinoid receptor (CB)-1
  - Cannabinoid receptor (CB)-2



- All receptors are widely expressed in the central nervous system and in peripheric tissues, like gut epithelium\* :
  - Enteric nervous system
  - Lymphocytes, macrophages, DC
  - Epithelial cells



\*Philippe D et al. Gut (Epub ahead of print), Massa F et al. J. Clin. Invest. 113, 1202-1209 (2004), Stein C et al. Nat Med 8, 1003-1009 (2003), Philippe D et al. J. Clin. Invest. 111, 1329-1338 (2003), D'Argenio G et al. FASEB. J. 20, 568-570 (2006).



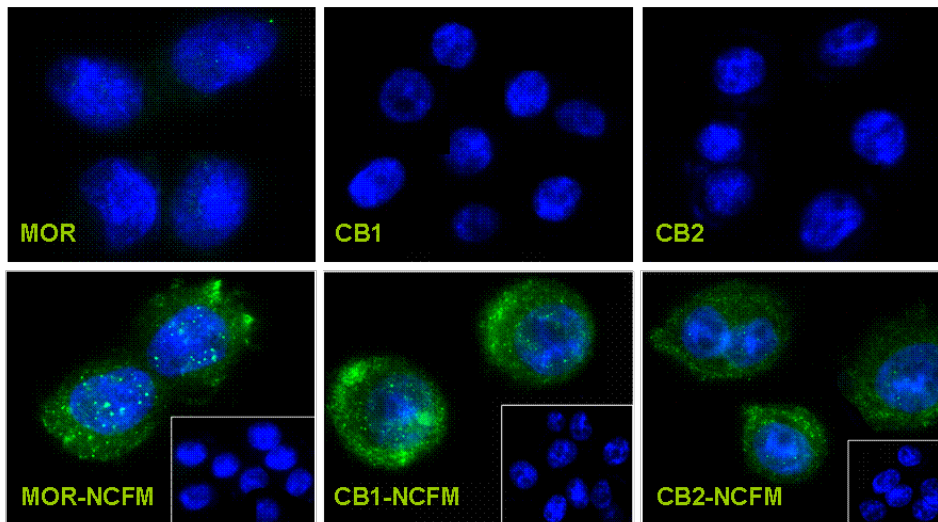
## *L. acidophilus* NCFM<sup>®</sup> can reduce gut pain – pre-clinical data

- Mechanism: modulation of pain-reducing receptor expression in the intestine
- Shows direct interaction between a probiotic and host nervous system receptors

nature  
medicine

*Lactobacillus acidophilus*  
modulates intestinal pain  
and induces opioid and  
cannabinoid receptors

Christel Rousseaux<sup>1-3</sup>, Xavier Thuru<sup>1-3,10</sup>, Agathe Gelot<sup>4-6,10</sup>,  
Nicolas Barnich<sup>7</sup>, Christel Neut<sup>1-3</sup>, Laurent Dubuquoy<sup>1-3</sup>,  
Caroline Dubuquoy<sup>1-3</sup>, Emilie Merour<sup>1-3</sup>, Karen Geboes<sup>8</sup>,  
Mathias Chamaillard<sup>1-3</sup>, Arthur Ouwehand<sup>9</sup>, Greg Leyer<sup>9</sup>,  
Didier Carcano<sup>9</sup>, Jean-Frédéric Colombel<sup>1-3</sup>, Denis Ardid<sup>4-6</sup> &  
Pierre Desreumaux<sup>1-3</sup>



Rousseaux et al., 2007:  
*L. acidophilus* NCFM induces  
expression of analgesic ('anti-pain')  
receptors in tissue culture cells

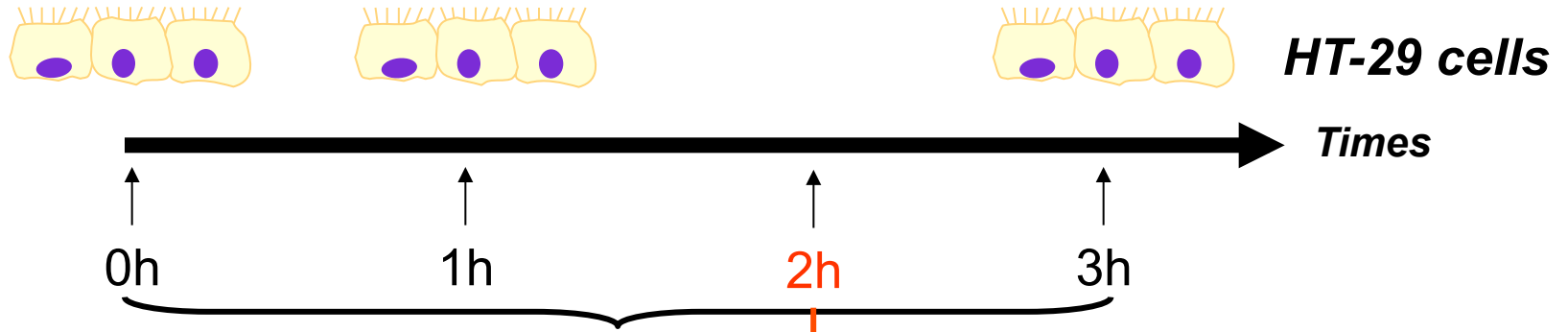
## **Aims of the study : In vitro experiments**

**To determine whether particular probiotic strains:**

**may induce expression of mu opioid (MOR) and cannabinoid 1 and 2 (CB1 and CB2) receptors on epithelial cells**

**and contribute to the modulation and restoration of normal visceral pain perception**

# Stimulation of human epithelial cells with probiotics and intestinal bacteria

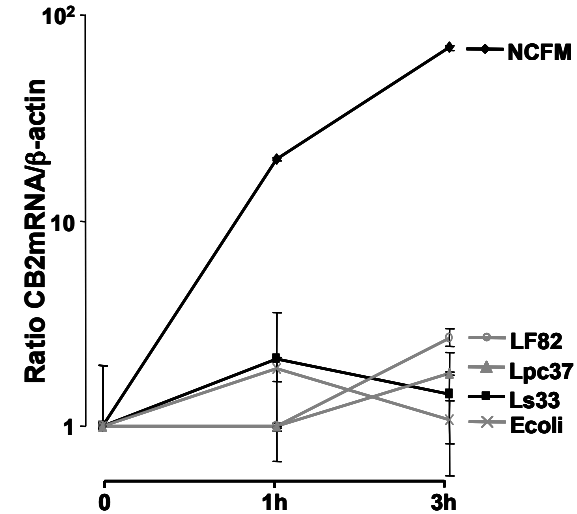
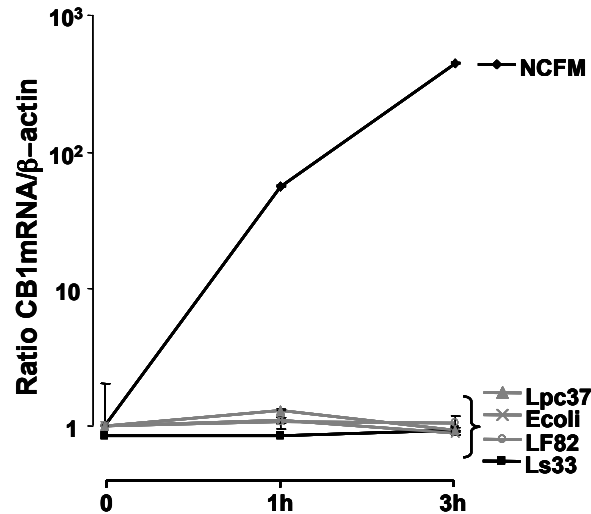
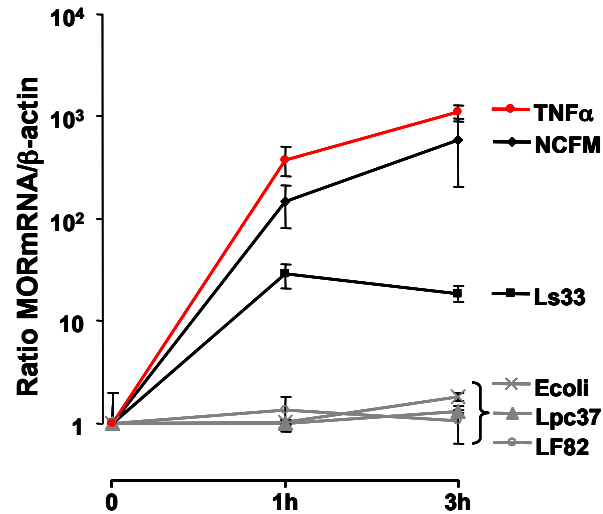


- **Lactobacillus (100 cfu/cell)**
  - L. acidophilus* NCFM
  - L. salivarius* Ls33
  - L. paracasei* Lpc37
- **Bifidobacterium (100 cfu/cell)**
  - B. lactis* Bi07
  - B. lactis* BI04
- **Escherichia coli (100 cfu/cell)**
  - Commensal (cEc)
  - Adherent-invasive (LF82)

**TNF $\alpha$  (10 ng/ml)\***

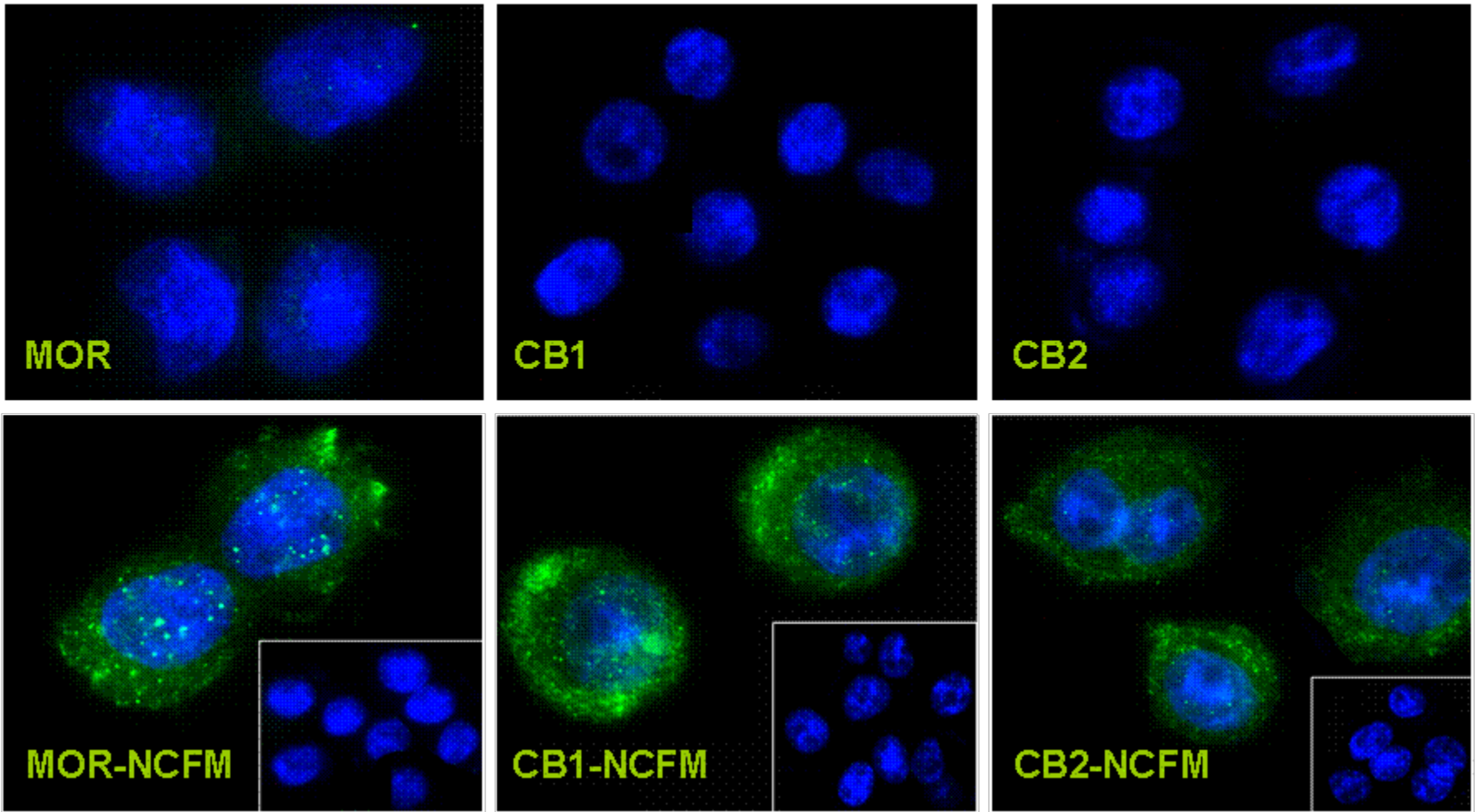
\*: [Philippe D et al. Gut](#) (Epub ahead of print)

# Only *L. acidophilus* NCFM strains induced significant expression of MOR, CB1 and CB2 mRNA by epithelial cells

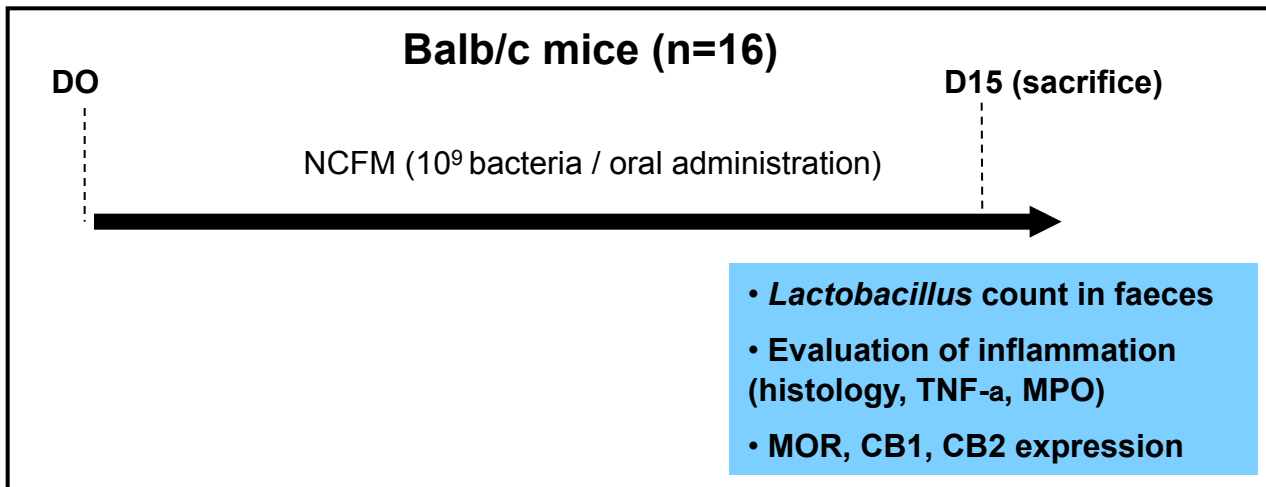


- *L. salivarius* (Ls33) induced MOR mRNA expression
- *L. paracasei* (Lpc37), *B. lactis* Bi07 and BL04 strains, and the two controls *E. coli* were ineffective

***L. acidophilus* NCFM strains induced expression of MOR, CB1 and CB2 protein by HT-29 epithelial cells**

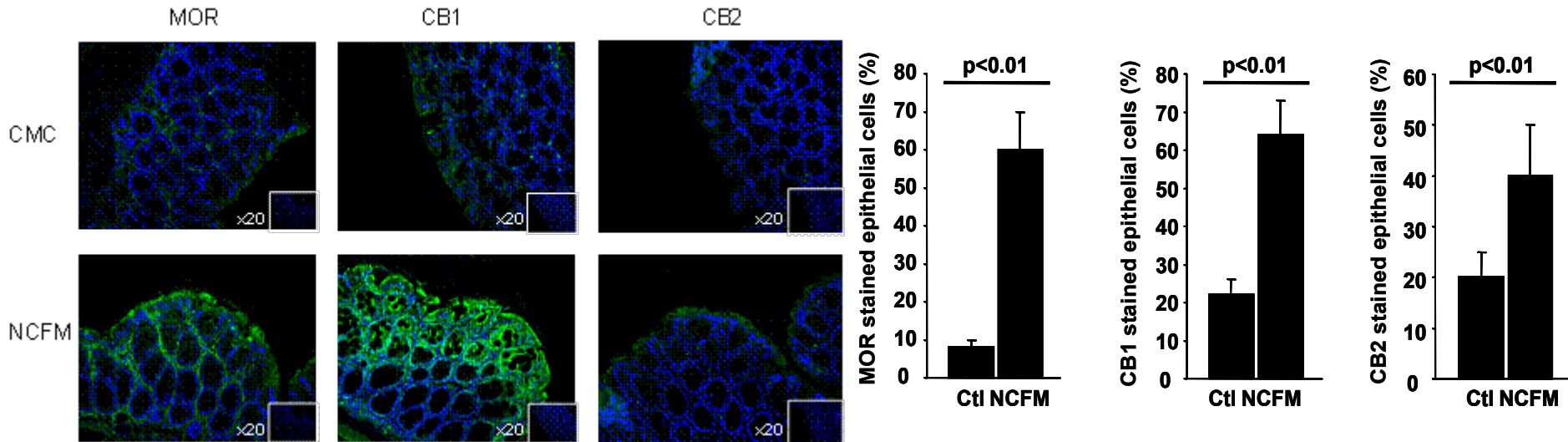


# NCFM induced MOR , CB1 and CB2 mRNA expression in vivo in mice

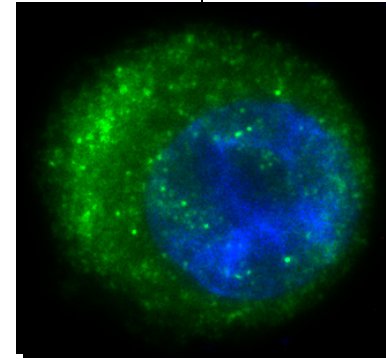
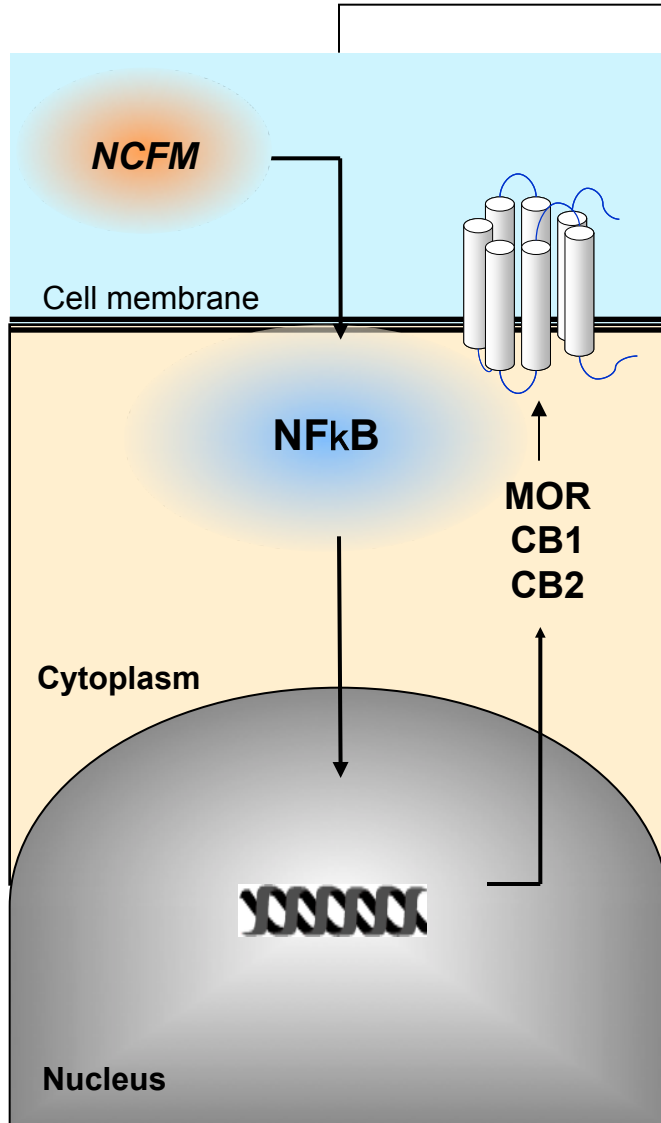


# NCFM induced MOR , CB1 and CB2 mRNA expression in vivo in mice

- No macroscopic, histologic inflammation in mice treated with NCFM
- No modification of MPO and TNF- $\alpha$  colonic concentrations in NCFM treated mice compared to untreated animals
- Induction of MOR, CB1 and CB2 expression in NCFM treated mice



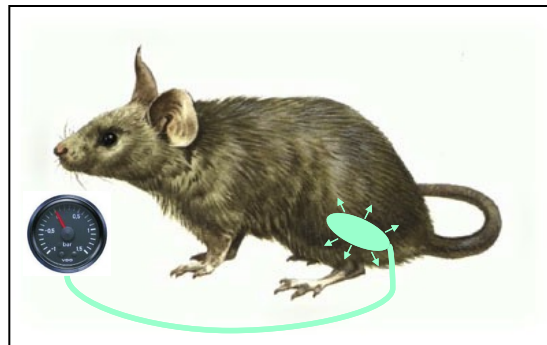
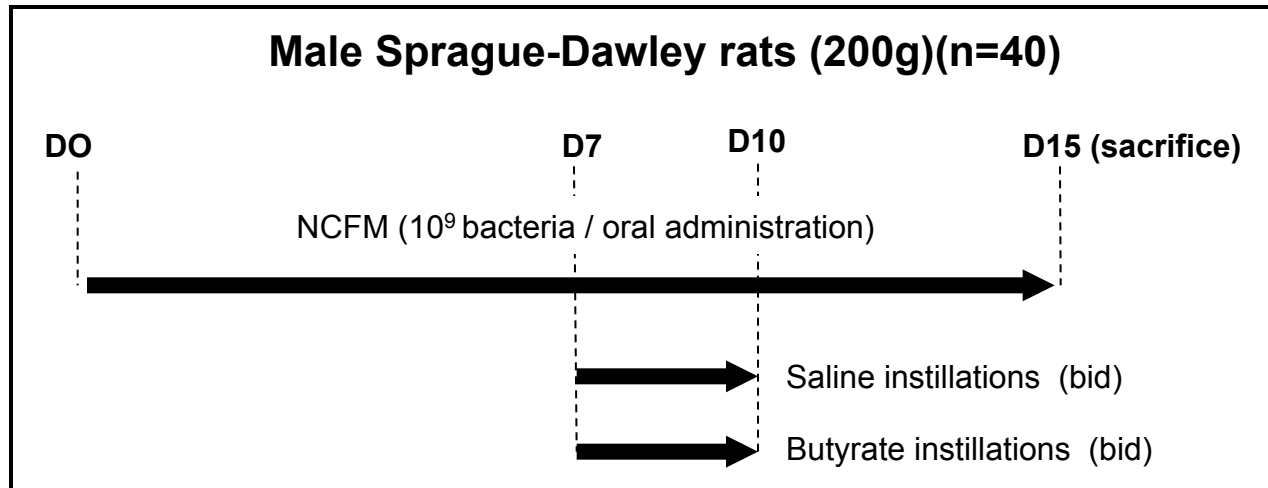
# NCFM strains induced MOR, CB1 and CB2 expression in epithelial cells through the NFkB pathway



*What is the functional role of NCFM-induced analgesic receptors ?*



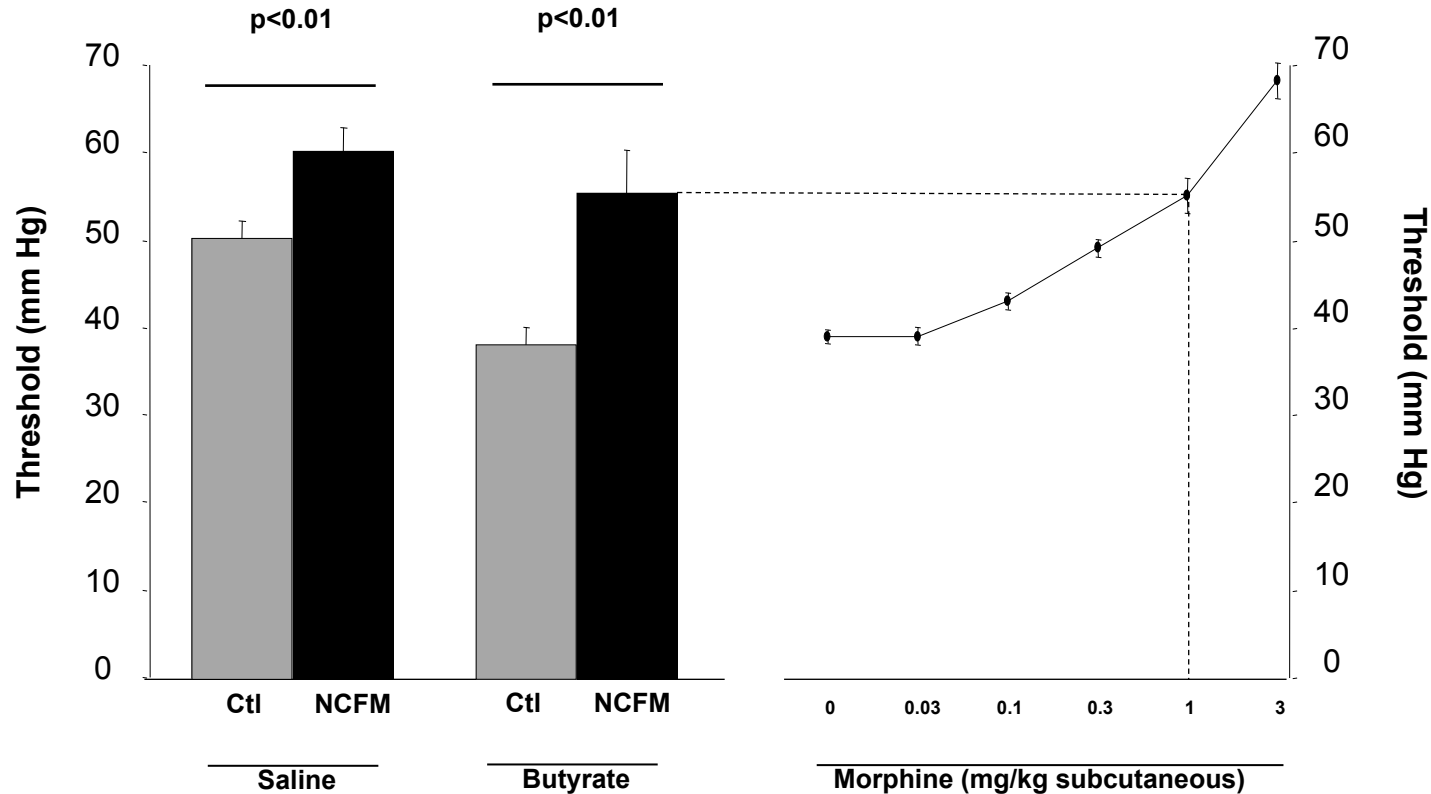
# Evaluation of the functional role of NCFM-induced analgesic receptors in rats measured by colorectal distension



*Colorectal distension after inflation of a balloon inserted intrarectally and connected to a barostat system\**

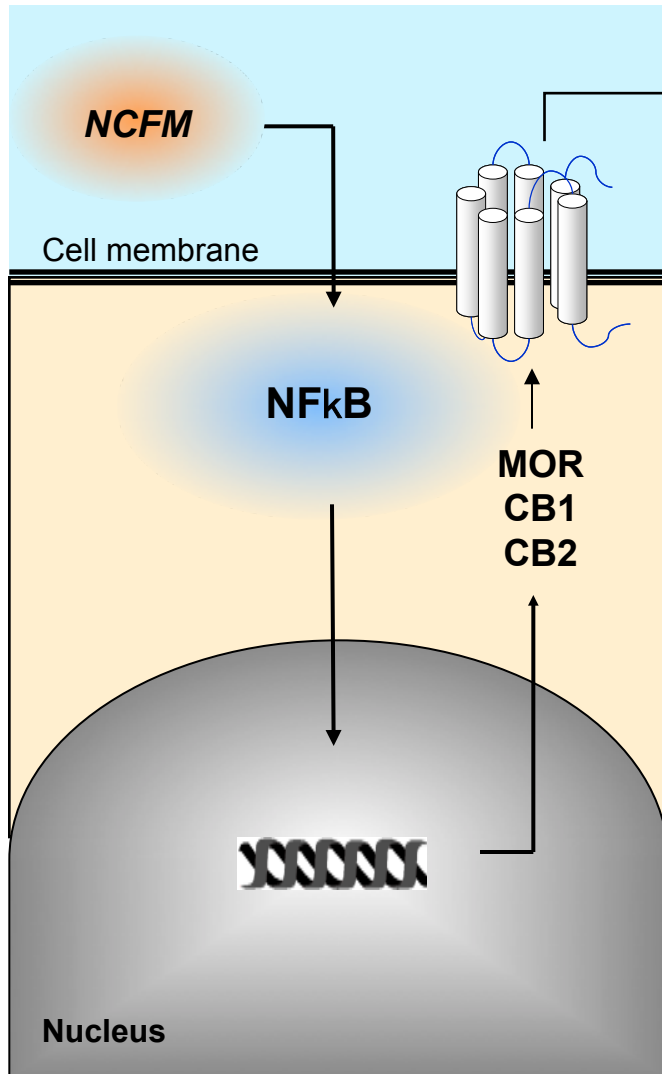
\*Verdu EF et al. Gut 55, 182-190 (2006), Kamiya T et al. Gut 55, 191-6 (2006). Bourdu S et al. Gastroenterology 128, 1996-2008 (2005)

# NCFM administration induced modulation and restoration of visceral pain perception



- NCFM decreased visceral perception allowing a 20% increase of pain threshold
- and a 44% increase of pain threshold in rat with colonic hypersensitivity
- NCFM mediated a similar effect than 1mg/kg of morphine s/c

# NCFM induces MOR, CB1, CB2 expression and mediates analgesic effect in the gut



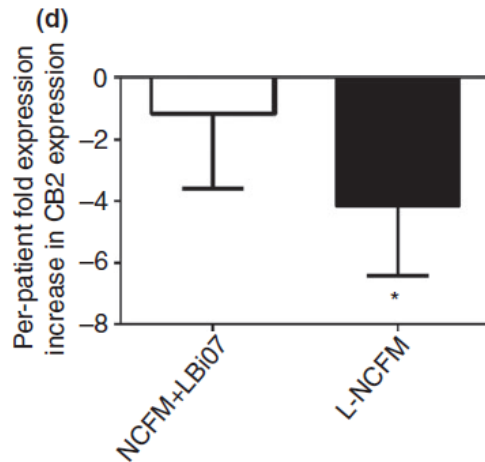
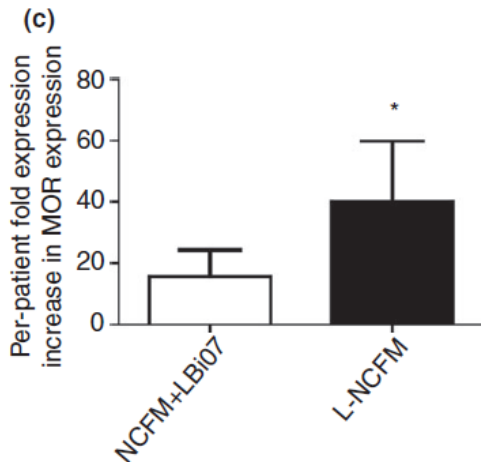
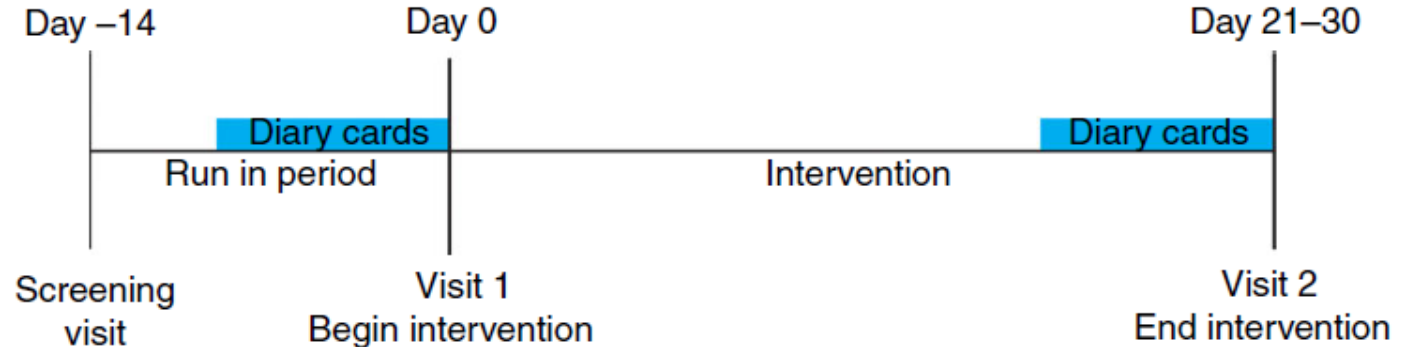
*NCFM increases pain threshold in the gut*

## CONCLUSION

*L. acidophilus* NCFM induces MOR, CB1, CB2 expression and mediates analgesic effect in the gut

## *Lactobacillus acidophilus* NCFM affects colonic mucosal opioid receptor expression in patients with functional abdominal pain - a randomised clinical study

T. Ringel-Kulka<sup>\*1</sup>, J. R. Goldsmith<sup>†1</sup>, I. M. Carroll<sup>†</sup>, S. P. Barros<sup>‡</sup>, O. Palsson<sup>†</sup>, C. Jobin<sup>†,§</sup> & Y. Ringel<sup>†</sup>



# Reduction of post-colonoscopy pain (D'Souza et al., 2015)

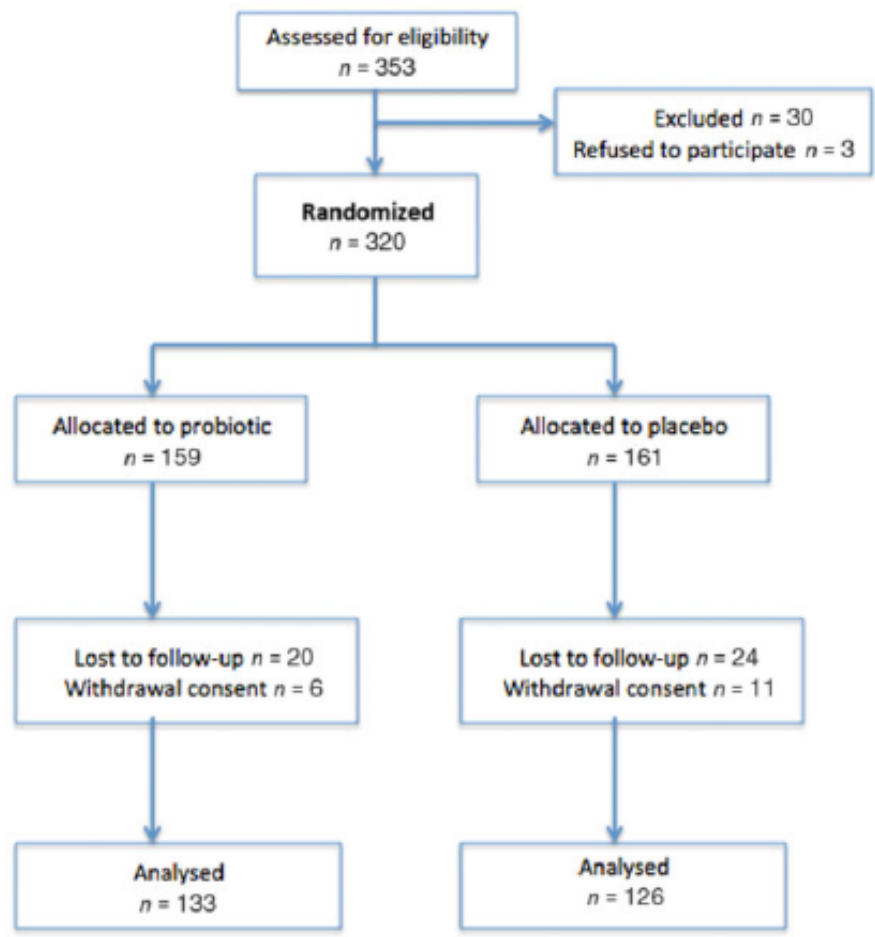


Enrolment

Allocation

Follow-up

Analysis



Probiotic:  
NCFM and Bi-07  $1.25 \times 10^{10}$  CFU each

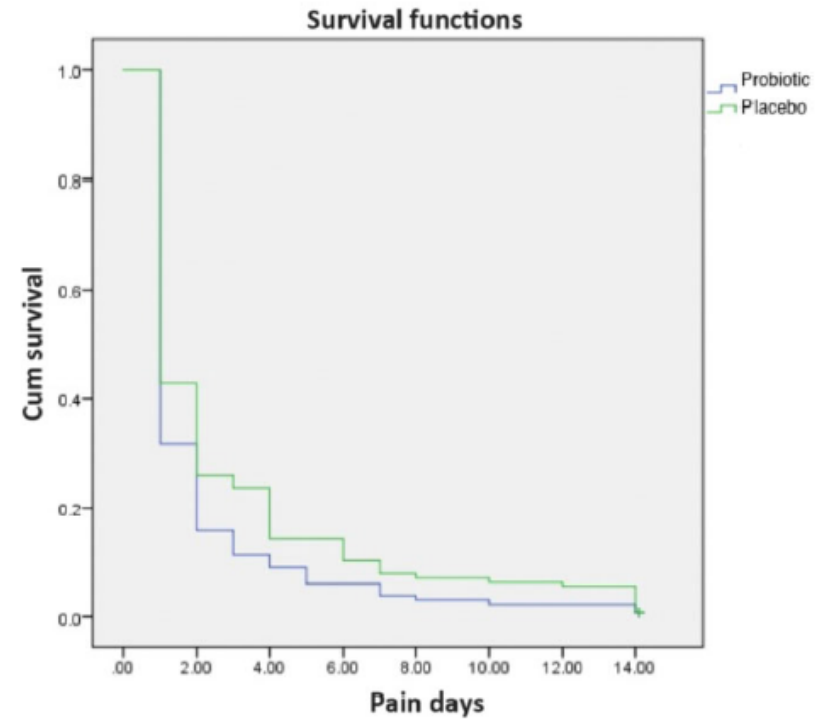


Fig. 2. Kaplan-Meier survival curve for differences in pain resolution between probiotic and placebo ( $P = 0.028$ ).

Variable	Statistic	Probiotic (n = 133)	Placebo (n = 126)	P value
Bloating	Mean	2.000	2.517	0.111
	Standard deviation	1.996	3.054	
Pain	Mean	1.993	2.779	0.032
	Standard deviation	2.398	3.361	
Return of normal bowel habit	Mean	3.054	3.422	0.280
	Standard deviation	2.198	3.156	

# Lactose intolerance symptoms

Reference	Subjects	Delivery format	Dose	Conclusion
Montes RG, et al. 1995. Effect of milks inoculated with <i>Lactobacillus acidophilus</i> or a yogurt starter culture in lactose-maldigesting children. J Dairy Sci. 78: 1657-1664.	20; 5-16 y	Milk containing probiotic	$10^{10}$ cfu/d	H <sub>2</sub> excretion not reduced but symptoms alleviated; different mechanism than in the case of regular yogurt starter cultures

20 lactose mal-digesting children (5-16 yr)

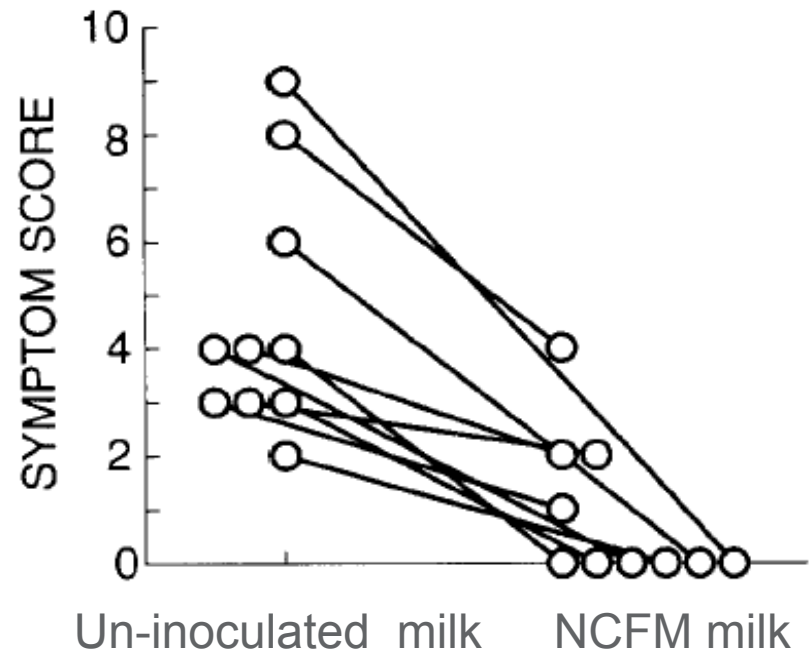
Single blinded study

Symptoms and breath H<sub>2</sub> excretion evaluated

$10^{10}$  cfu NCFM® in milk (11.6g lactose)

Compared to:

- $10^{10}$  *S. thermophilus*
- Plain milk



Combined symptom score of abdominal pain, bloating, gas, cramps, flatus, abdominal rumbling

## To conclude

Functional bowel disorders common – Visceral pain a common symptom

- Visceral allodynia and hyperglasia may sensitize to pain

Increased gut permeability, low-level inflammation, microbial imbalance can induce sensitization to intestinal pain

- Probiotics may counteract these

*L. acidophilus* NCFM can directly influence the expression of pain-relieving receptors in the gut

- Pre-clinical study demonstrating mechanism-of-action and clinical data confirming the effect



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