

Butyrate produced by gut commensal bacteria activates *TGF- β 1* expression through the transcription factor SP1 in human intestinal epithelial cells.

Camille Martin-Gallausiaux^{1,2}, Fabienne Béguet-Crespel¹, Ludovica Marinelli^{1,2}, Alexandre Jamet¹, Florence Ledue¹, Hervé M. Blottière^{1,3} and Nicolas Lapaque^{1*}.

1. Micalis Institute, INRA, AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France;

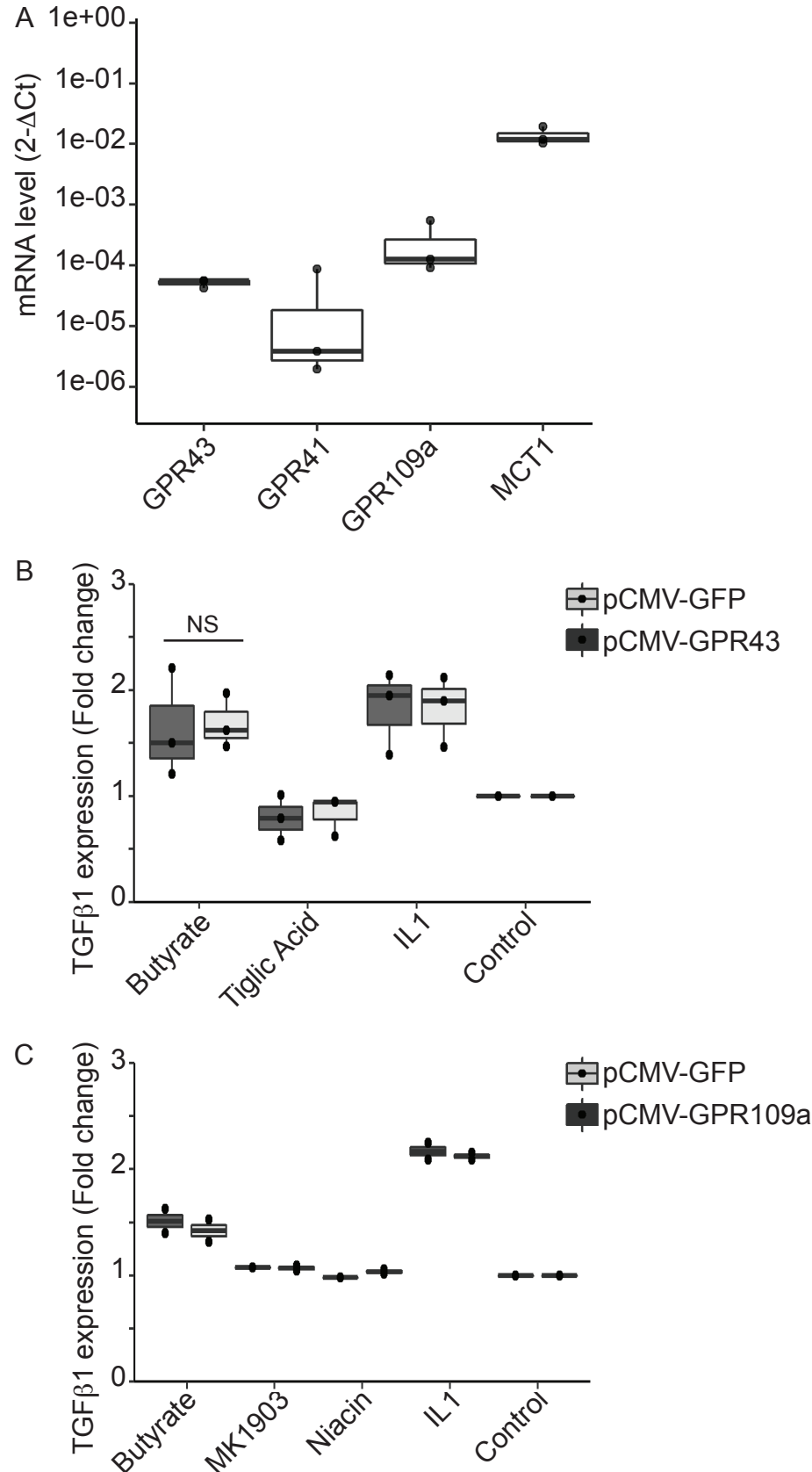
2. Sorbonne Université, Collège Doctoral, F-75005 Paris, France.

3. MetaGenoPolis, INRA, Université Paris-Saclay, 78350 Jouy en Josas, France.

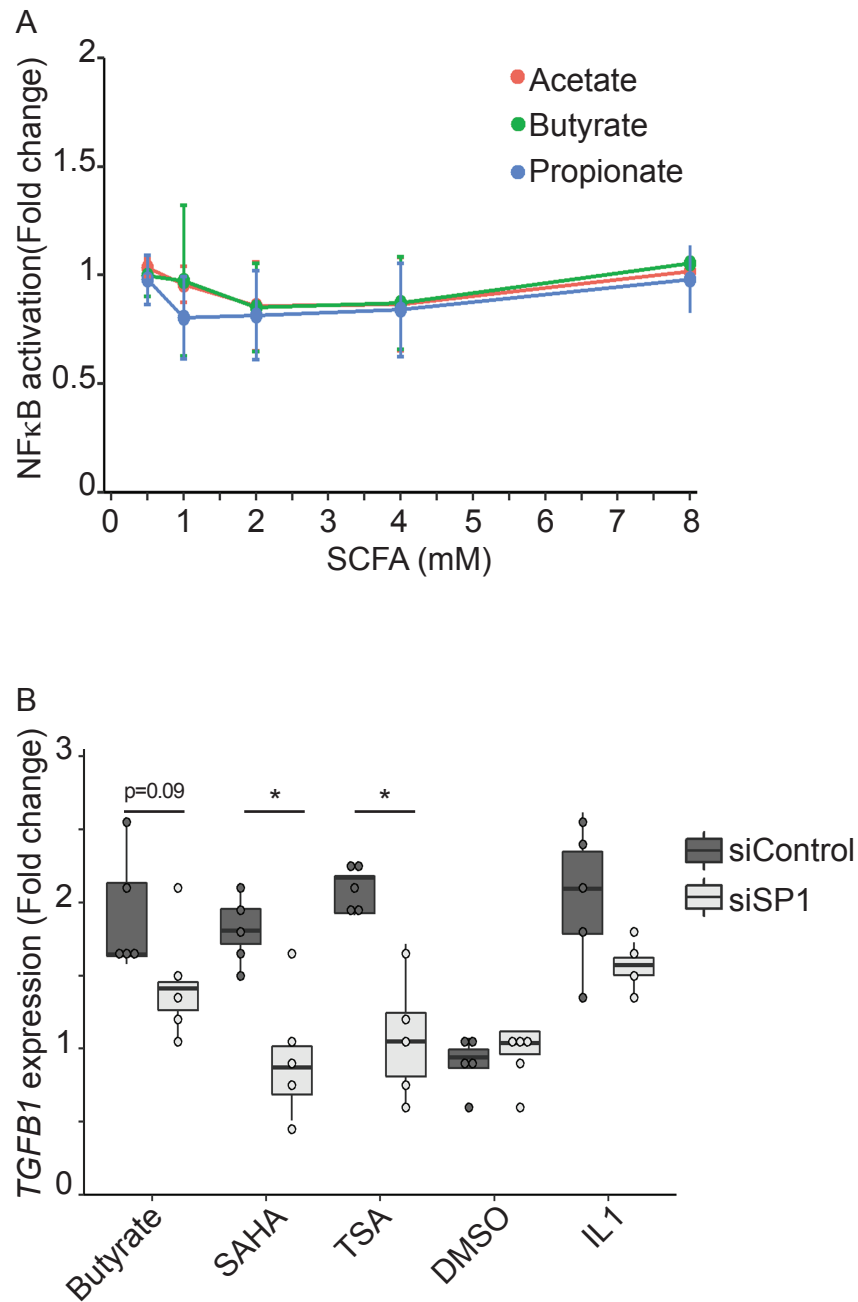
* Corresponding author: Dr Nicolas Lapaque

INRA-MICALIS UMR1319, Bat 442, Domaine de Vilvert 78350, Jouy-en-Josas, France.

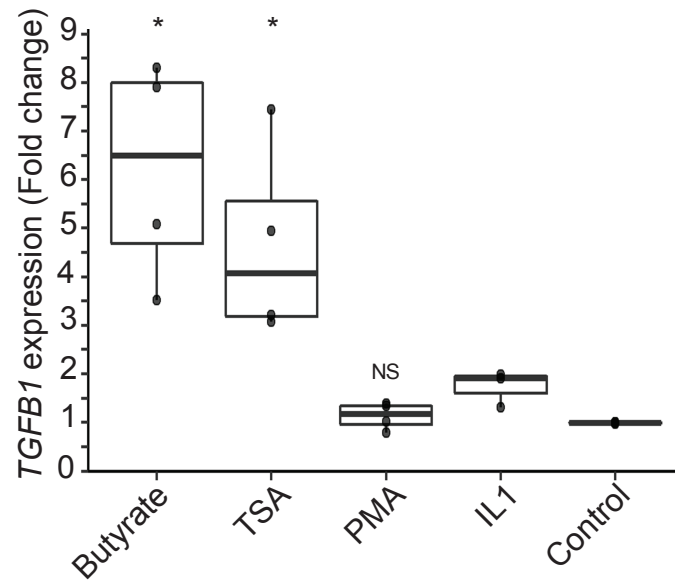
e-mail: nicolas.lapaque@inra.fr



Supplementary Figure S2: Butyrate mediated impact on *TGFB1* is independent of its receptors GPR41, GPR43, GPR109a. (A). Expression of the G-protein coupled receptors *GPR41*, *GPR43*, *GPR109a* and *MCT1* in HT-29. Gene expression was determined by quantitative real-time PCR on total RNA extracted from HT-29. HT-29- TGF_{prom} reporter cells were transfected with pCMV-FFAR2 (*GPR43*), N=3 (B) and pCMV-HACR2, (*GPR109a*) N=2 (C) prior to the incubation for 24h with IL1 β (10ng/ml), butyrate (2mM) or with the GPR agonists (B) Tiglic acid (10mM) (C): Niacine (10mM) and MK1903 (1 μ M). *TGFB1* expression was measured by luciferase activity and expressed as median $\pm$ quartiles of fold change towards un-stimulated cells, t-test, *P<0.05, **P<0.01, ***P<0.001.



Supplementary Figure S3: (A) HT-29-NFκB reporter cells were incubated for 24h with different concentrations of butyrate. NF-κB activation was measured by SEAP secretion and expressed as fold increase towards un-stimulated cells, N=3. (B) HT-29-TGF_{prom} reporter cells were transfected with SP1 siRNA or control siRNA and incubated with butyrate (2mM), TSA (1μM), SAHA (5μM) or IL1β (100U/ml) for 24h.. *TGFB1* expression was measured by luciferase activity and expressed as median ± quartiles of fold change towards un-stimulated cells. Wilcoxon test, N=5, *P<0.05, **P<0.01, ***P<0.001.



Supplementary Figure S4: HT-29-TGF $_{-453/+11}$ cells with double AP-1 mutations were treated with Butyrate (4mM), TSA (1 μ M), IL1 β (10ng/ml) or PMA (100nM) for 24h. *TGFB1* expression was measured by luciferase activity and expressed as median $\pm$ quartiles of fold change towards un-stimulated cells. Wilcoxon test, N=4, *P<0.05, **P<0.01, ***P<0.001.