

Jejunum-derived NF- κ B reporter organoids as 3D models for the study of TNF- α -induced inflammation.

Hellen Daghero, Flora Doffe, Belén Varela, Victoria Yozzi, José Manuel Verdes, Martina Crispo, Mariela Bollati-Fogolín and Romina Pagotto

Supplementary Materials

Comparison of TNF- α -induced reporter response from different intestinal region-derived organoids.

Organoids derived from duodenum, jejunum, ileum and colon were obtained from the transgenic reporter mice NF- κ B hRE-Luc. They were stimulated with different concentrations of TNF- α and 24 h later, luciferase activity was measured as the mean of NF- κ B activation. When possible, data presented in **Figure 4b** from the manuscript were fit to a 3 parameters dose-response curve using GraphPad Prism software. Results showed that jejunum-derived organoids best fit the model, with an R square of 0.916 and an EC50 of 3.66 ng/mL (Table S1).

Regarding the reporter response elicited by TNF- α 10ng/mL, jejunum and ileum derived-organoids response was statistically different from colonoids (ANOVA and Tukey's multiple comparison-test; *P< 0.05), which were unresponsive to the stimulus (Figure S1).

Parameter	Duodenum	Organoids derived from Jejunum	Ileum	Colon
EC50	12.11	3.66	0.12	-
CI 95%	1.64-8.16	0.98-14.9	very wide	-
R2	0.54	0.92	0.47	-

Table S1: TNF- α concentration-response curve fitting parameters from different intestinal region-derived-organoids. The half-maximal effective concentration (EC50) to TNF- α , the corresponding 95% confidence interval (CI 95%) and the R square of the curve is indicated for each type of organoid. Data was obtained from triplicates from one representative experiment.

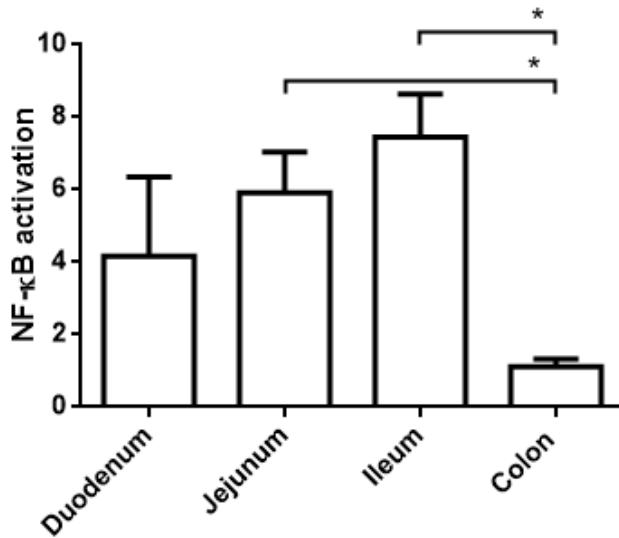


Figure S1: Comparison of the reporter response of different intestinal region-derived-organoids to TNF- α 10 ng/mL. Data was expressed as mean \pm SD of triplicates from one representative experiment. * $P < 0.05$, significant differences in the NF- κ B activation level attained between the indicated type of organoids.

Comparison of TNF- α -induced reporter response from the same intestinal region-derived organoids.

Duodenum-, jejunum-, ileum- and colon-derived organoids obtained from different NF- κ B hRE-Luc mice ($n=3$ for each region) were stimulated with TNF- α 10 ng/mL and 24 h later, luciferase activity was measured. Results showed that jejunum and ileum responses to TNF- α were significantly higher compared to the unstimulated organoids (ANOVA and Tukey's multiple comparison-test; # $P < 0.05$). Nevertheless, the maximum level of NF- κ B activation attained was different between animals (ANOVA and Tukey's multiple comparison-test; * $P < 0.05$). Two organoids did not differ from the control value regarding duodenum, whereas colon-derived organoids were unresponsive in all cases (Figure S2).

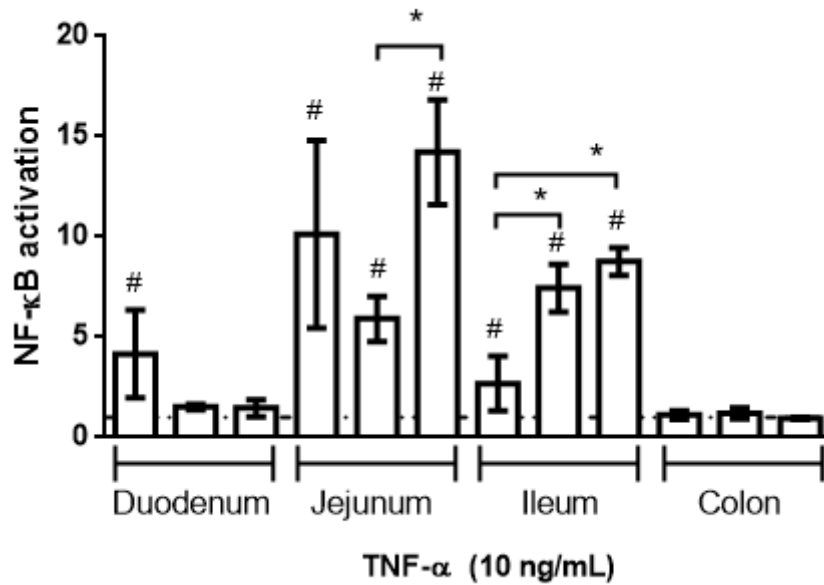


Figure S2: Comparison of the reporter response of organoids from different animals to TNF- α 10 ng/mL. Organoids derived from different NF- κ B hRE-Luc mice were stimulated with TNF- α 10 ng/mL and the reporter response was measured. NF- κ B activation was expressed as fold change with respect to the unstimulated control. Data was expressed as mean \pm SD of triplicates. *P < 0.05, significant differences between indicated bars; #P < 0.05, significant differences with respect to the unstimulated control from the same intestinal region-derived organoid.

Gene (mouse)	Forward Primer	Reverse Primer	NM number
Chromogranin A	5'AGACTACAGACCCACTCCCG 3'	5'AGATGACTTCCAGGACGCAC 3'	NM 007693.2
Lgr5	5'TCTCCTACATCGCCTCTGCT 3'	5'TTCCTCCGGAACCTGTCTCA 3'	NM 010195
Lysozyme	5'AACTACAACCGTGGAGACCG 3'	5'TGCAATTGATCCCACAGGCA 3'	NM 0.13590.4
Mucin 2	5'AACTGCTCTCTGGACTGCC 3'	5'TTGGTTGGTGTGCTGAGTGT 3'	NM 023566.3
Villin	5'ATTCCTTCAGACGCATGCCA 3'	5'GCCCTCCCTTTTGAGTGTGA 3'	NM 009509.2
β-actin	5'GCAGGAGTACGATGAGTCCG 3'	5'ACGCAGCTCAGTAACAGTCC 3'	NM 007393.5
TNF-R1/ Tnfrsf1a	5'GATCCCCTGCCTGTCAAAGA 3'	5'TCGCAAGGTCTGCATTGTCA 3'	NM 011609.4
TNF-R2/ Tnfrsf1b	5'GATGCCAAGGTGCCTCATGT 3'	5'TGGCTTCCGTGGGAAGAATC 3'	NM 011610.3

Table S2: Primers used for real-time RT-PCR experiments