ANSWERING REVIEWERS

The authors want to thank the reviewers for their positive comments on our work. We believe that the present manuscript has been consistently improved by the reviewers' observations. We made any effort to accomplish all the proposed suggestions. Corrections have been highlighted in red. A detailed response follows.

To the Editor: we made all the indicated corrections for the format of the manuscript. We could not find the certificate for the grant approval, so we deleted the grant number from the funds acknowledgement. Since that grant was not specifically restricted to the present work, we believe that the indication of "Public Funds from "Sapienza" University of Roma (Italy)" would correctly indicate that part of the source of the funds. We did not include ARRIVE guidelines and Institutional Animal Care and Use Committee Approval Form, since they are not applicable for the present work for we did not use animals for the experiments.

To Reviewer 1: the authors want to thank the reviewer for the positive comment on our work. We tried to address all the indications, in particular:

- English grammar has consistently revised by an English professional editing service (American Journal Experts).
- Title was modified as suggested
- Abstract length was reduced and the p values included as indicated
- "proof-the-concept" has been changed with "poof-of concept"
- Introduction was modified as suggested, and sentences specifically focusing on IBD has been added (from line 10).
- The ethic committee was named
- In the Discussion section supplementary sentences focusing of evidence for post-biotic effect of LGG products has been included (page 15, line 15) and a suggested reference has been added (ref n. 35).

To Reviewer 2: the authors want to thank the reviewer for the positive comments on the present paper. We tried to address all the raised observations, in particular:

- Two sentences referring to the possible risks of probiotics and LGG administration have been added in the Introduction (Page 5, line 17) and in the Discussion sections (Page 16, line 20), and two extra references have been added (ref n. 9 and 37)
- All species' names have been written in italics
- RPMI composition has been specified
- Numbers have been written in scientific format as indicated
- No specific primers has been developed for LGG to date for SybrGreen dye based detection system for RT-PCR, so we used primers targeting general Lactobacillus

species. We agree with the reviewer that other lactobacilli could be unspecifically detected by the methodology and cannot be excluded, but the consistent increase of concentration in relatively short time (2h incubation in the *ex-vivo* experiment, a week in the *in vivo* investigation), and the fact that we restricted our investigation to the mucosa and not to the feces (that are probably more easily affected by exogenous probiotics administration), suggest that the increase observed by RT-PCR is more likely ascribable to LGG. We added in the Discussion section a paragraph addressing that issue (page 14, from line 1).

- We double-checked the reported statistical significances. The statistical evaluation of data presented in Figure 3 (including 3.0±0.3 vs. 3.7±0.3) has been performed with t test for paired samples, since the data refers to paired observations. We specified that in the Results section, adding "for paired samples" to the reported p value.
- Typing and grammar errors have been corrected by revision from a professional editing service (American Journal Experts). Style of reference n. 23 (n.24 in the revised version of the manuscript) has been modified.

To Reviewer 3: the authors want to thank the reviewer for the positive comments and the important suggestions. Since no specific data on adhesion and mucosal effect of LGG in colonic mucosa are available to date, in the present preliminary proof-of-concept study we focused on the analysis of the single species (LGG) and on its regulatory effect on two of the main pro-inflammatory cytokines (i.e. TNFα and IL-17). We completely agree with the reviewer that the evaluation of expression and production of different Th1 and Th2 cytokines, as well as the confirmation of our findings with different methodologies (i.e. FISH or electron microscopy), would be of great interest, and some of the proposed investigations are still being performed at our institution. The preliminary findings of our work can open the way to a more in-depth investigation of the effect of LGG and to the comparison with other probiotic species. We addressed that issue in the Discussion section (pag.15, line 15). We further tried to address the reviewer suggestions and in particular:

- Novel findings have been better highlighted in Abstract and Discussion section
- Merit and limitation of short-term organ culture have been addressed in Discussion section (pag.13, from line 20).
- The final sentence in Abstract section has been removed as suggested
- Incubation time for the experiments has been set after evaluation of different time-points. We specified that in the Materials and Methods section (pag.8, line 4)
- We agree that trends reported may simply reflect a type 2 statistical error, and we stated that in the Discussion section (page 13, line 13)
- Figure 3 has been modified by adding a third panel (panel C) showing TNF expression in LGG CM-incubated and control biopsies from normal colon.