frontiers

Supplementary Material

Morphological and functional characterization of IL-12Rβ2 chain on intestinal epithelial cells: implications for local and systemic immunoregulation

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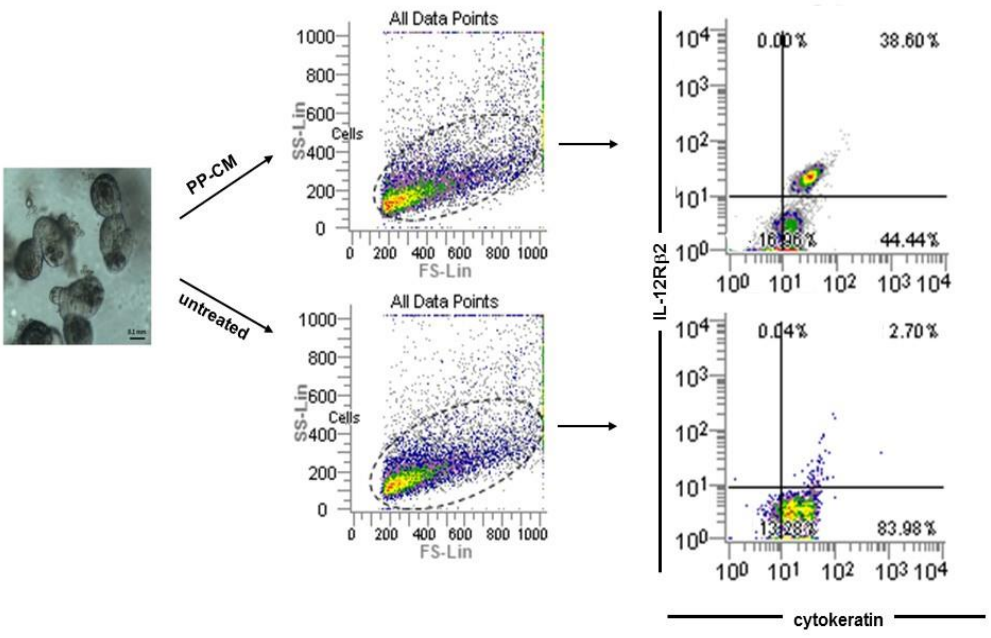
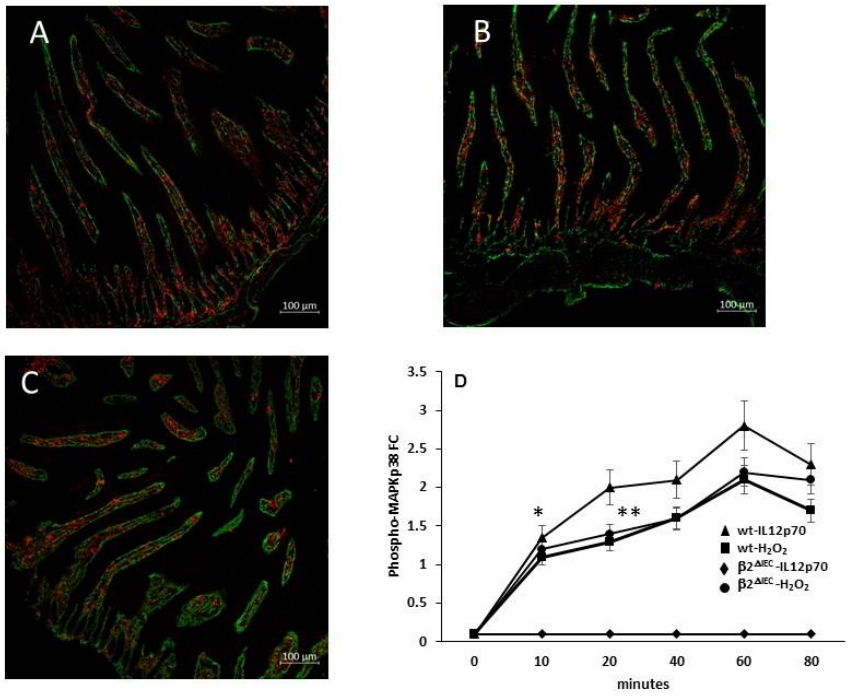
1 Supplementary Figures and Tables



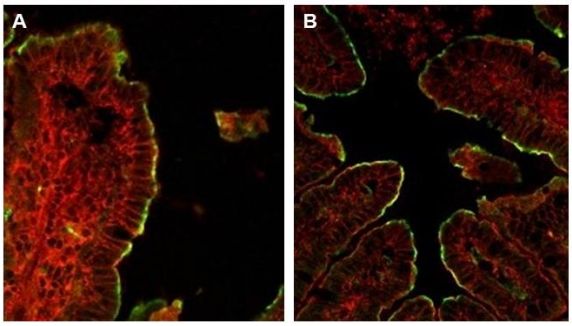
Supplementary Figure 1. **Expression of IEC-associated IL-12Rβ2 chain is regulated by signals derived from immune cells.** Primary intestinal organoids were developed *in vitro*. In order to evaluate the effects of immune system-derived signals on the expression of the IL-12Rβ2 chain on IECs culture

medium was supplemented with conditioned medium collected from 3-day culture of Peyer's patch-derived immune cells (PP-CM) or left not supplemented (untreated). Following the preparation of cells suspension the cells were analyzed by flow cytometry. Organoid intestinal epithelial cells grown in the presence of PP-CM showed a significantly higher proportion of organoid cells expressing the IL-12Rβ2 chain compared to cell grown in normal not supplemented culture medium. Furthermore, the effect of the PP-CM is twofold. Indeed, in addition to the increase of the percentage of cells expressing the IL-12Rβ2 chain, these cells also showed an increased expression of the epithelial marker cytokeratin. Two batches of intestinal organoids/treatment were prepared and samples were run in triplicate.



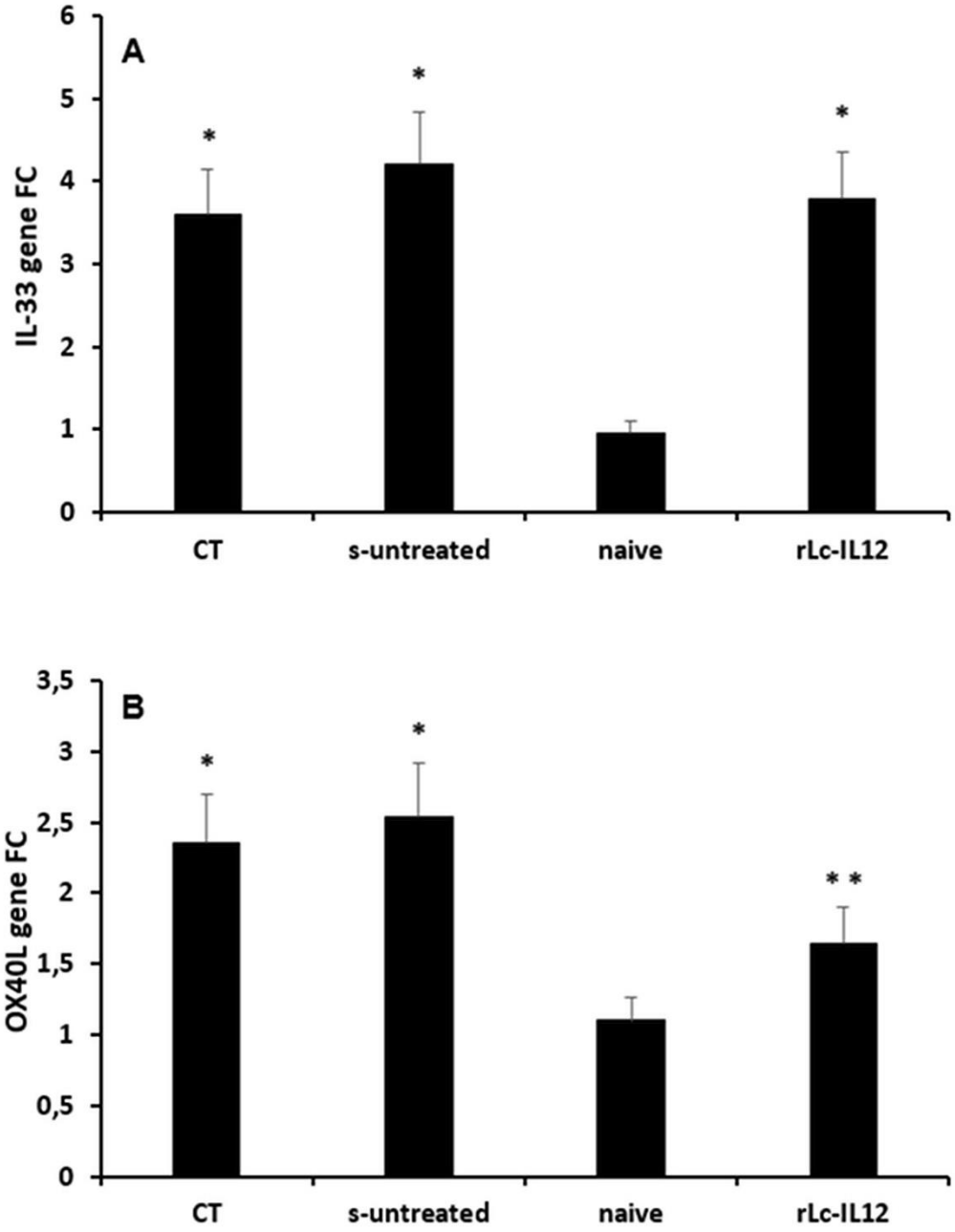
Supplementary Figure 2. **Post BM-transplant levels of MHC II^{+} cells in the intestinal tissue**. Wild type (A) and chimeric IL-12Rβ2$^{-/-}$ (BM from IL-12Rβ2$^{-/-}$ → IL-12Rβ2$^{-/-}$) recipient mice) (B) and IL-12Rβ2$^{\Delta IEC}$ (BM from wt → IL-12Rβ2$^{-/-}$ recipient mice) (C) were monitored for the levels of MHCII^{+} cells (red) using rat anti-I-A/I-E(clone M5/114.15.2, Biolegend) within the lamina propria (lp) on week 8 post BM-transplant. Sections were counterstained (green) with rabbit anti-entactin antibody (Abcam). Immunofluorescence analysis revealed similar levels of expression of MHCII^{+}cells in the lp thus showing a successful engraftment. These analysis complemented the functional experiment carried out as described in Figure 4 of the main text. Furthermore, IECs from both wild type and IL-12Rβ2$^{\Delta IEC}$ mice were isolated and challenged *in vitro* with bioactive IL-12p70; levels of phospho-MAPKp38 were then evaluated by flow cytometry. In agreement with what observed in Figure 1F for wt and IL-12Rβ2$^{-/-}$ mice

we observed that also IECs from chimeric IL-12Rβ2$^{\Delta IEC}$ (β2$^{\Delta IEC}$-IL12p70) did not respond to IL-12. By contrast, these cells responded when exposed to a non-specific activation stimulus (H_2O_2). (*) indicates statistical difference between wt-derived IEC challenged with both IL-12p70 or H_2O_2 and β2$^{\Delta IEC}$-derived IEC challenged with H_2O_2 compared to β2$^{\Delta IEC}$-derived IECs challenged with IL-12p70. (**) indicates statistical difference between wt-derived IECs challenged with IL-12p70 and both wt- and β2$^{\Delta IEC}$-IECs challenged with H_2O_2. Experiments were performed in triplicate using freshly isolated IECs from 3 mice/group.



Supplementary Figure 3. Immunofluorescence staining with anti-TSLP antibody in the gut of chimeric mice. Immunofluorescence staining carried out in both IL-12Rβ2$^{-/-}$ (A) and IL-12Rβ2$^{\Delta IEC}$ (B) chimeric mice undergoing allergic sensitization showed detectable levels of TSLP (green) in the epithelium following oral delivery of recombinant bacterial vector producing IL-12p70 (rLc-IL12). Sections were counterstained (red) with mAb anti-β-actin antibody (Clone AC15) (Sigma-Aldrich,

Milan, Italy). These data complemented data in Figure 7B-E of the main text and confirm that the presence of IL-12Rβ2 plays a role, among other factors in the control of TSLP production by IECs**.**



Supplementary Figure 4. Effects of rLc-IL12 on the intestinal expression of IL-33 and OX-40L. Mice treated with cholera toxin (CT) and in those sensitized by administration of CT in combination with allergen (s-untreated) and not treated with rLc-IL12 induced a significant increase of the expression of IL-33 in the intestinal epithelium compared to control naive mice; further, levels of IL-33 were not

affected by treatment with rLc-IL12 (A). Furthermore (B), also intestinal levels of OX40L were significantly up-regulated in CT and sensitized (s-untreated) mice compared to control naïve (*); however, in this case oral administration of rLc-IL12 significantly down-regulated the expression of OX40L but not completely abolished it and it remained significantly higher than in naïve mice (**) . Transcript analysis was carried out in replicate mice (4-6 mice/group) in two independent assays. Data was analysed using a Student's t-test followed by application of the Bonferroni correction the level of which was set at $p \leq 0.0125$. $P<0.05$.