



Supplementary Figure 5. Treg analysis in the murine LI (related to Figures 5 and 6). (A) Gating strategy for the flow cytometric analysis of Tregs. After exclusion of cell debris, doublets and dead cells, CD45⁺ leukocytes were gated according to the shown sequence to determine the proportion of CD3⁺CD4⁺FoxP3⁺ Tregs within cell suspensions prepared from the lamina propria of murine colons. (B) Immunofluorescence staining for Tregs on LI tissue samples. Tregs were identified by the presence of (i) a nuclear FoxP3 signal co-localizing with DAPI signal, (ii) cytoplasmatic CD4 signal and (iii) cytoplasmatic CD3 signal. Left panel, overview. Scale bar, 100 μ m. Upper right panels, enlarged version of dotted zoom-out showing two Treg cells fulfilling all three criteria. Scale bars, 10 μ m. Lower right panels, individual signals of the same cells in the CD3, FoxP3 and CD4 channels. Scale bars, 10 μ m.