

Development of a preclinical model to study the influence of intestinal epithelium-ECM crosstalk

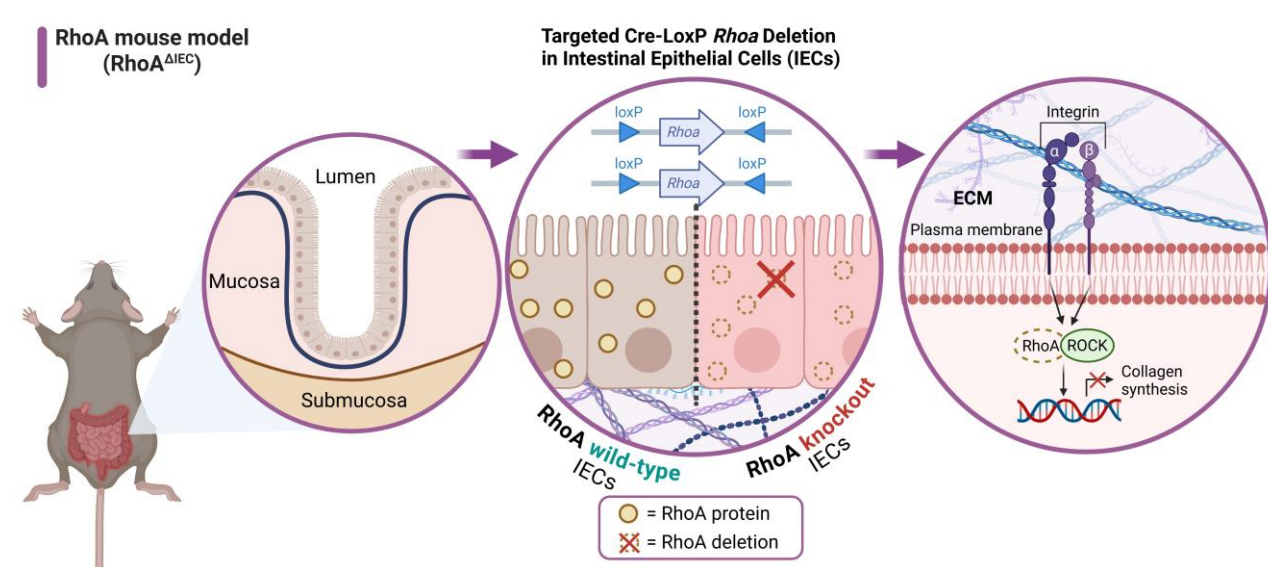
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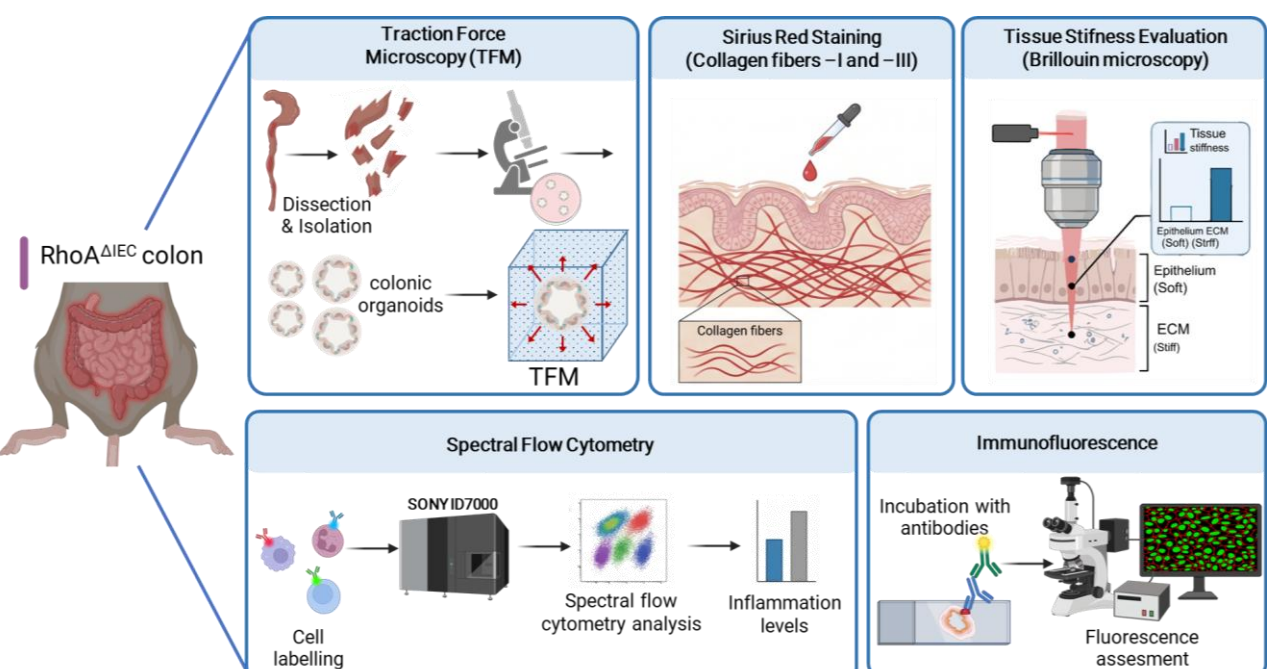
INTRODUCTION

The extracellular matrix (ECM) is a dynamic structure that, beyond its classical role as a tissue scaffold, actively regulates key biological processes and contributes to pathological contexts (1). Nevertheless, the specific role of ECM remodelling in intestinal disease progression and its relationship with epithelial barrier dysfunction remains largely unexplored (2). Here, we investigate how epithelial dysfunction drives ECM remodelling and whether these changes precede or follow immune cell infiltration and represent a mechanism to interfere with pathogenesis in intestinal diseases, such as Inflammatory Bowel Disease (IBD) and cancer (3).

METHODS



Development of RhoA^{ΔIEC} mouse model (4). To study the influence of epithelial dynamics and alterations in the ECM, we took advantage of a mouse model that carries a deletion in the cytoskeletal protein RhoA in the intestinal epithelium and leads to epithelial barrier impairment and an alteration in the tissue dynamics (Figure created with BioRender).



Used methods and techniques. Traction force microscopy was used to quantify cellular forces in organoids isolated from these mice (5). Additionally, we evaluated the collagen-fiber deposition (Sirius red staining), the proximal colon tissue stiffness by Brillouin microscopy and the level of inflammation (IF and FACs) (Figure created with BioRender and FigureLabs).

RESULTS

Figure 1. RhoA-deficient organoids exert lower traction forces than wildtype controls

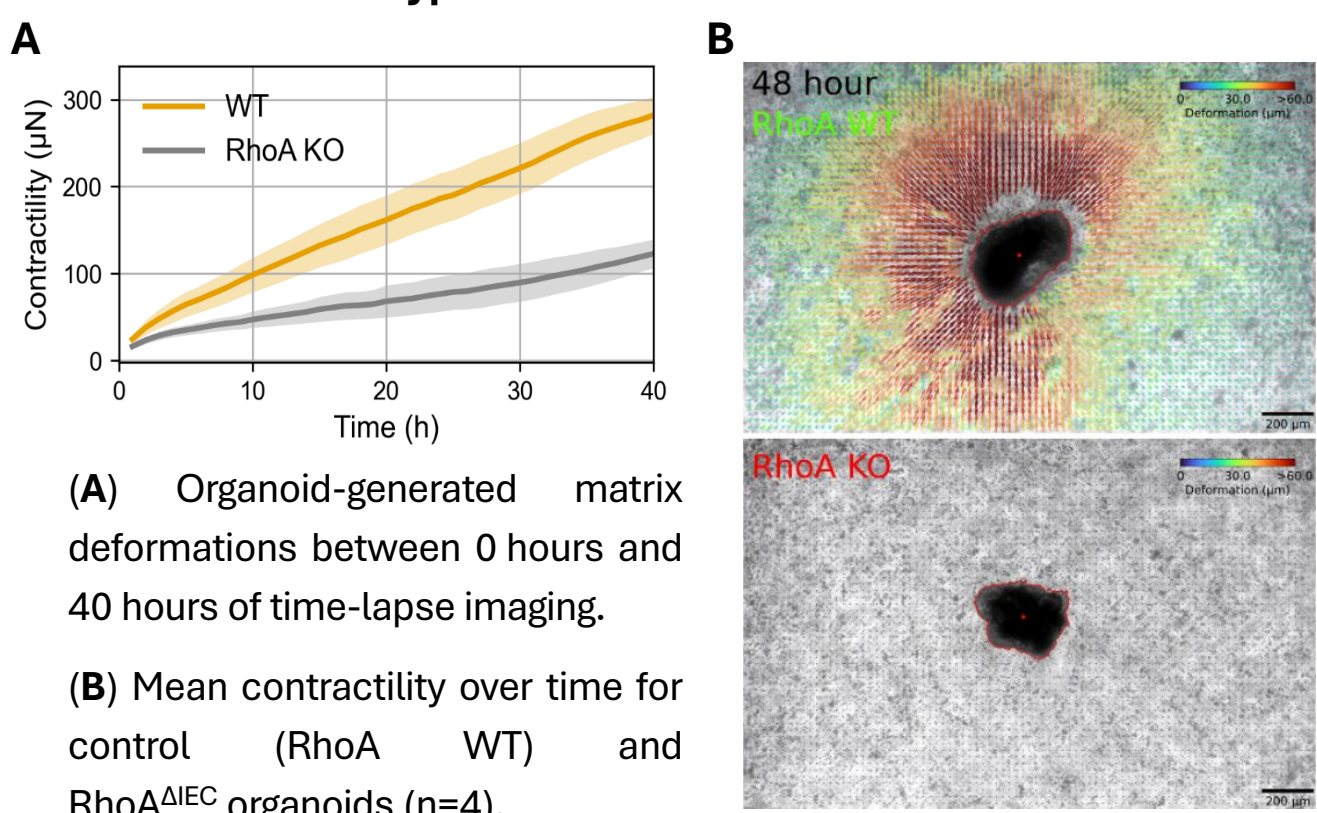
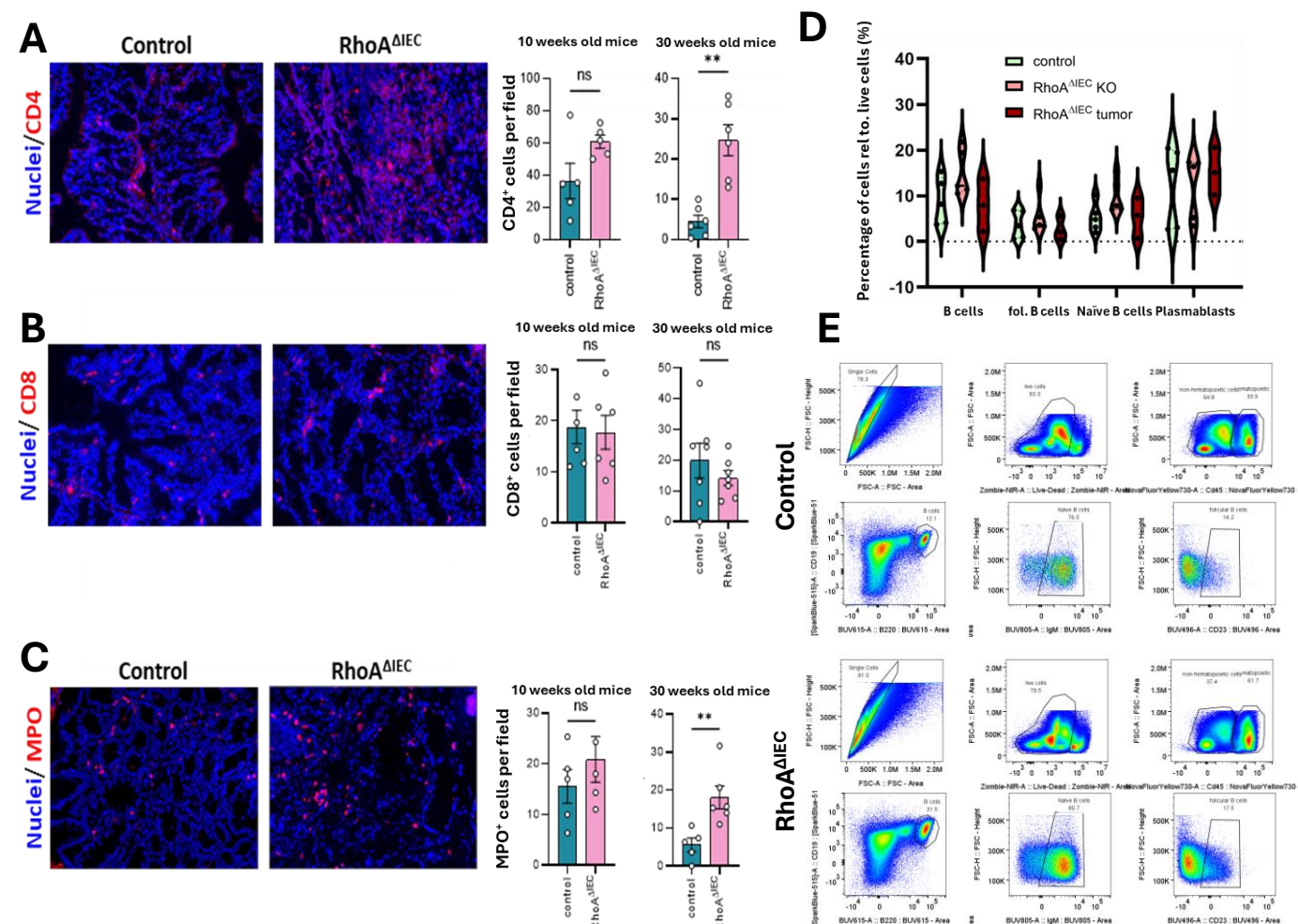
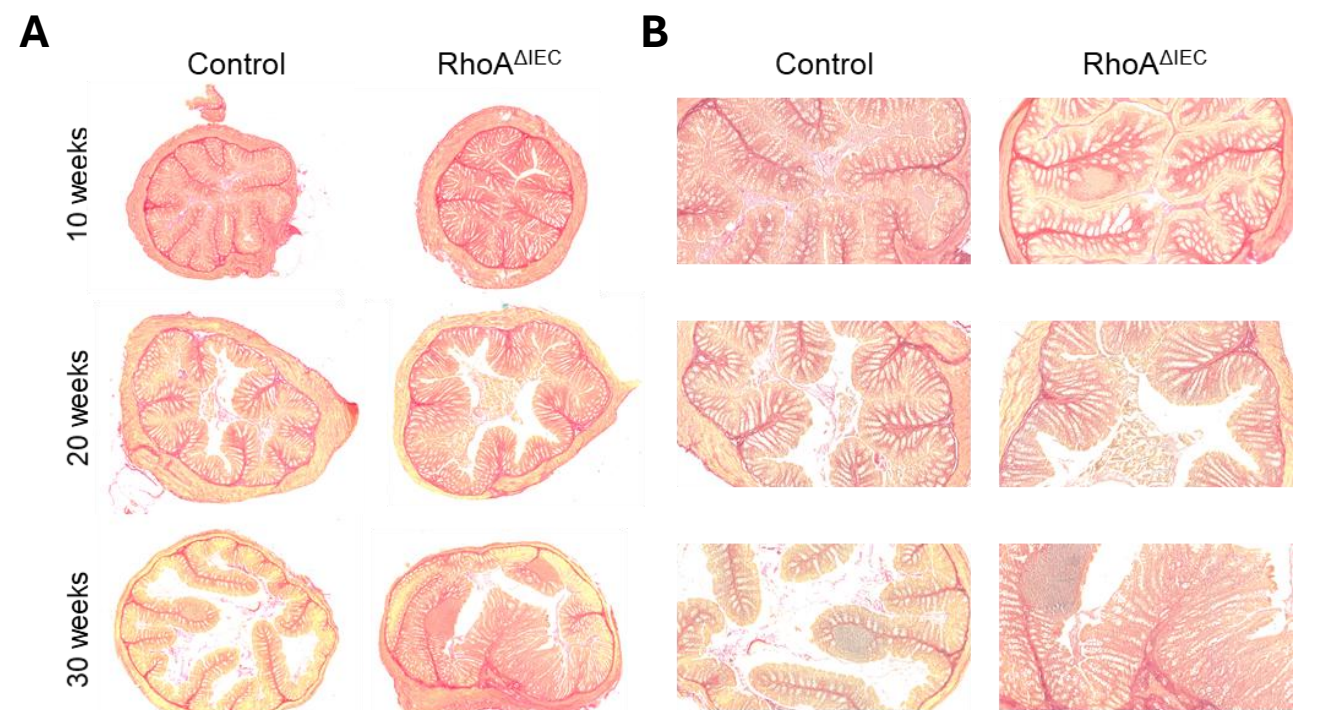


Figure 2. RhoA^{ΔIEC} mice show an altered infiltration of immune cells



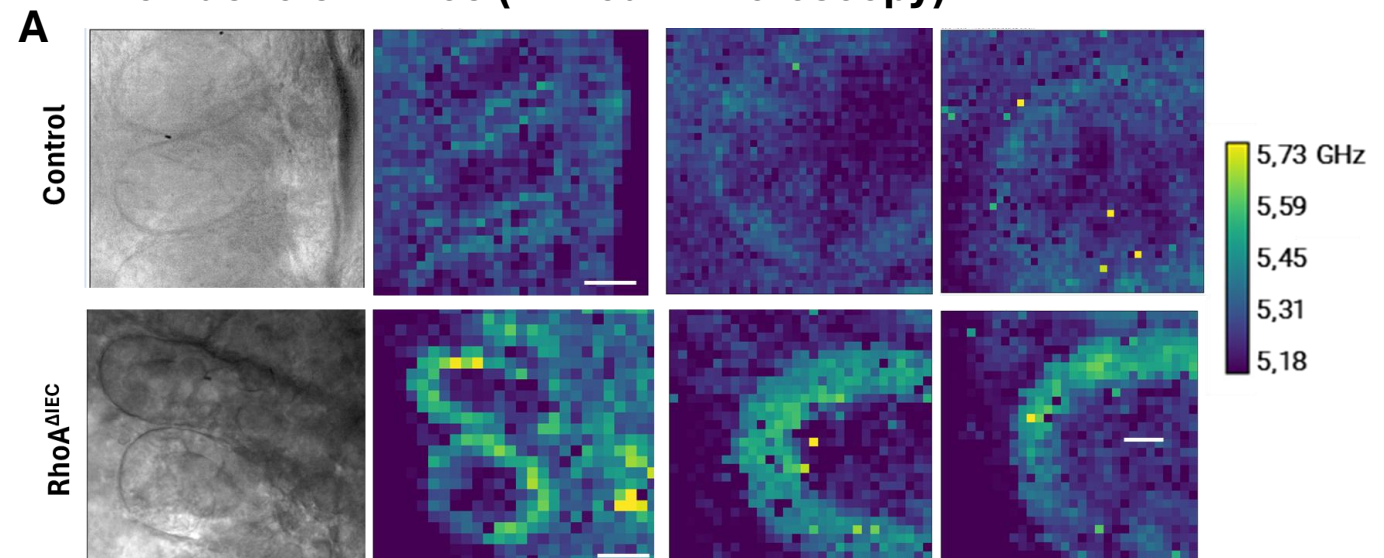
(A) CD4, (B) CD8 (C) MPO (neutrophils) immunofluorescence staining in cross-sections from proximal colon. Representative pictures (left), and corresponding quantification (right). (D) B cell counts in 40 weeks old RhoA^{ΔIEC} mice (spectral flow cytometry) and (E) representative gatings.

Figure 3. RhoA^{ΔIEC} mice show more collagen deposition.



(A, B) Representative Sirius images (n=4) of proximal colon from control and RhoA^{ΔIEC} mice (10, 20 and 30 weeks of age).

Figure 4. Measurement of the tissue stiffness in control and RhoA deficient mice (Brillouin microscopy)



(A) Representative Bright field (left) and Brillouin microscopy (right) images of proximal colon from control (above) and RhoA^{ΔIEC} (below) mice (40 weeks of age) (n=3).

CONCLUSION

Collectively, these findings suggest that changes in epithelial integrity due to RhoA deficiency result in altered traction forces, collagen deposition and associated immune infiltration. Nevertheless, their effects on the mechanical properties of the ECM still remain unclear.

REFERENCES

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