

Oral administration of CXCL12-expressing *Limosilactobacillus reuteri* improves colitis by local immunomodulatory actions in preclinical models

Emelie Öhnstedt,^{1,2} Cristian Donas,¹ Kristel Parv,¹ Yanhong Pang (presenter),¹ Hava Lofton Tomenius,^{1,2} Macarena Carrasco Lopez,¹ Venkata Ram Gannavarapu,² Jacqueline Choi,² Maria Ovezik,² Peter Frank,¹ Margareth Jorvid,¹ Stefan Roos,³ Evelina Vågesjö,^{1,2} and Mia Phillipson^{2,4}



¹Ilya Pharma AB, Uppsala, Sweden; ²Division of Integrative Physiology, Department of Medical Cell Biology, Uppsala University, Uppsala, Sweden; ³Department of Molecular Sciences, Swedish University of Agriculture, Uppsala, Sweden; and ⁴The Science for Life Laboratory, Uppsala University, Uppsala, Sweden

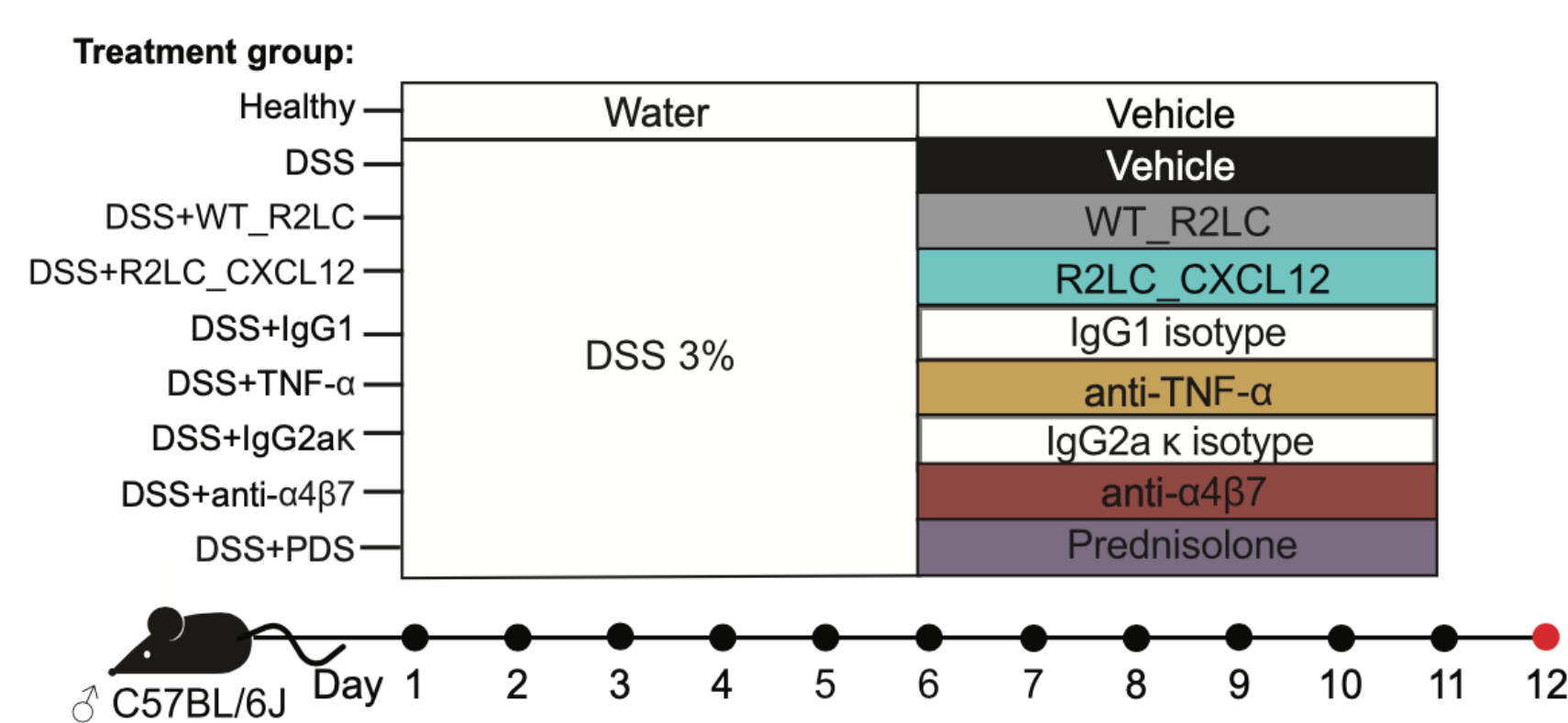


INTRODUCTION

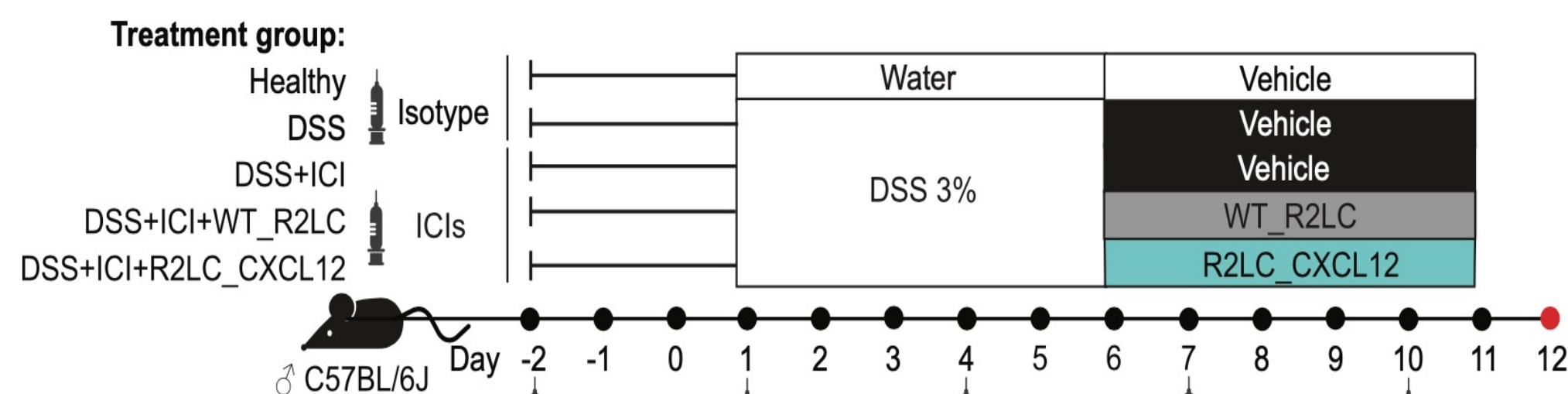
- Current colitis treatments rely on systemic immune suppression, alternatives for local effects also cause severe systemic adverse events; true localized treatments promoting mucosal healing without immune compromise are needed.
- Approximately 1.7 million cancer patients undergoing treatment with immune checkpoint inhibitors (ICIs) have been diagnosed with clinically evident ICI-induced colitis.
- The study aimed to develop local immunotherapy using genetically modified *L. reuteri* to deliver CXCL12, enhancing mucosal healing in DSS- and ICI-induced colitis models without impacting the immune system systemically.

METHODS

DSS-model

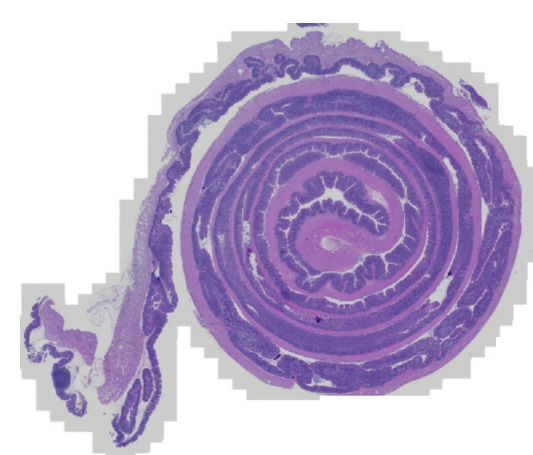


DSS-ICI-model



For the two methods above, oral gavage (3 doses/day) with vehicle, WT_R2LC, or R2LC_CXCL12, or i.p. injections (1 dose/day) of anti-TNF or anti-α4β7 or 1 dose/3 days

Pathology and immunohistochemistry

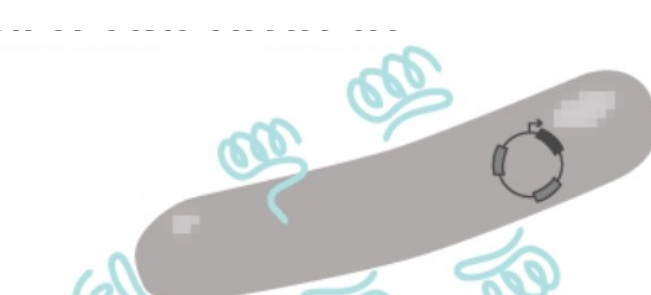


Ileum and colon Swiss rolls were prepared from mice were analyzed using pathology scoring and antibodies against immune cells and healing biomarkers

SUMMARY

Peroral CXCL12-producing *L. reuteri* R2LC efficiently ameliorates colitis, enhances mucosal healing, and has a favourable safety profile

ILP100 - emilogene sigulactibac

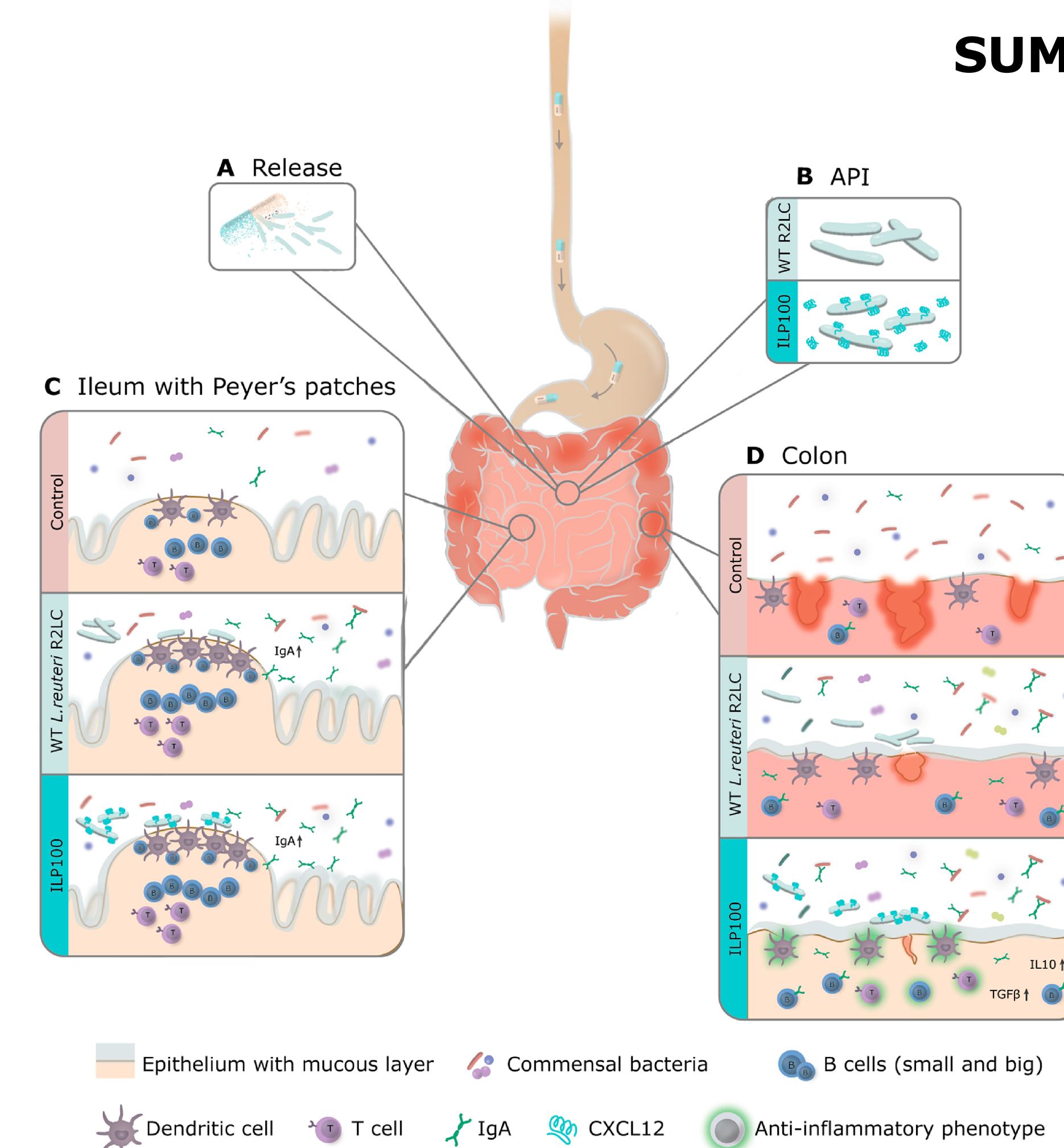


Genetically modified *Limosilactobacillus reuteri* R2LC transformed to express CXCL12

ILP100 – Drug Product



Lyophilized ILP100 in capsules releasing the contents in neutral pH



R2LC_CXCL12 ameliorates inflammation during overt colitis by increasing the colonic populations of anti-inflammatory immune cells, and demonstrates outperforming treatment efficacy comparable to established therapies

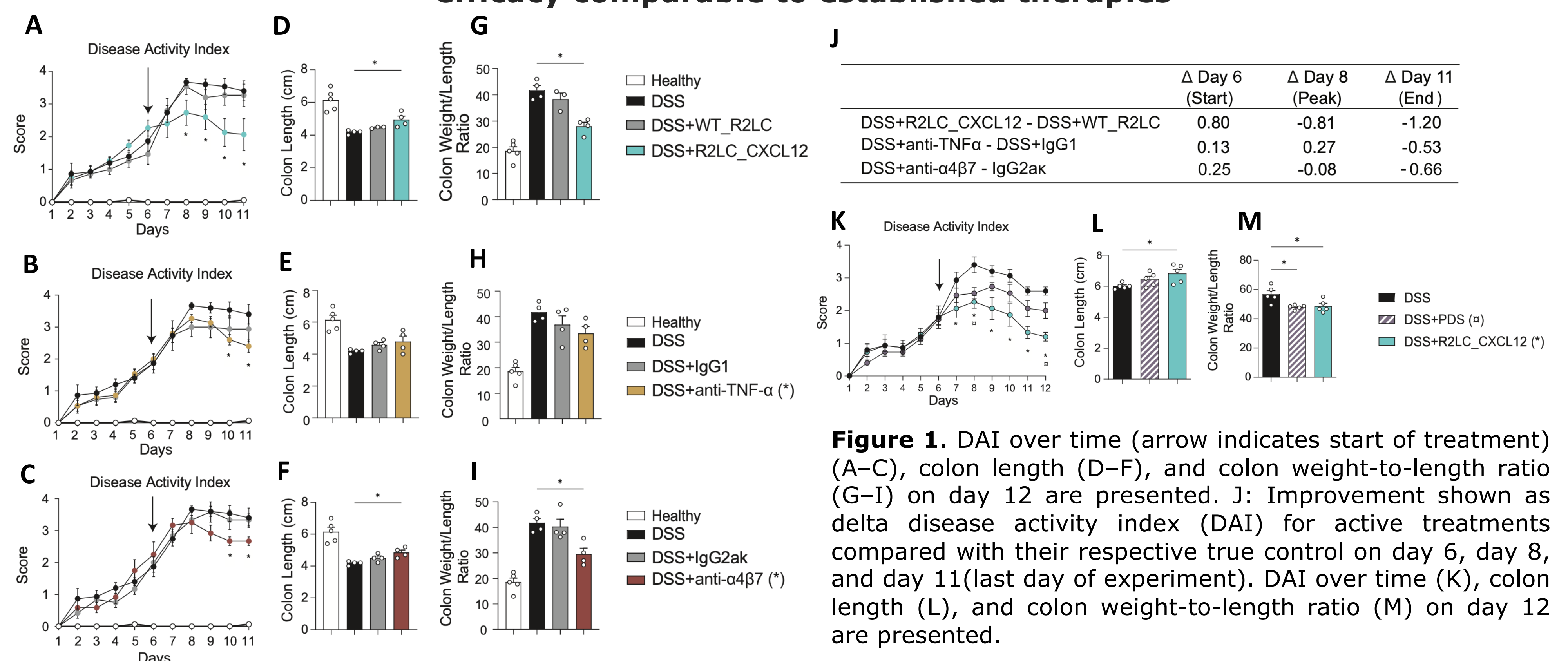


Figure 1. DAI over time (arrow indicates start of treatment) (A–C), colon length (D–F), and colon weight-to-length ratio (G–I) on day 12 are presented. J: Improvement shown as delta disease activity index (DAI) for active treatments compared with their respective true control on day 6, day 8, and day 11 (last day of experiment). DAI over time (K), colon length (L), and colon weight-to-length ratio (M) on day 12 are presented.

R2LC_CXCL12 ameliorates colitis symptoms in a mouse model of immune checkpoint inhibitor (ICI)-colitis

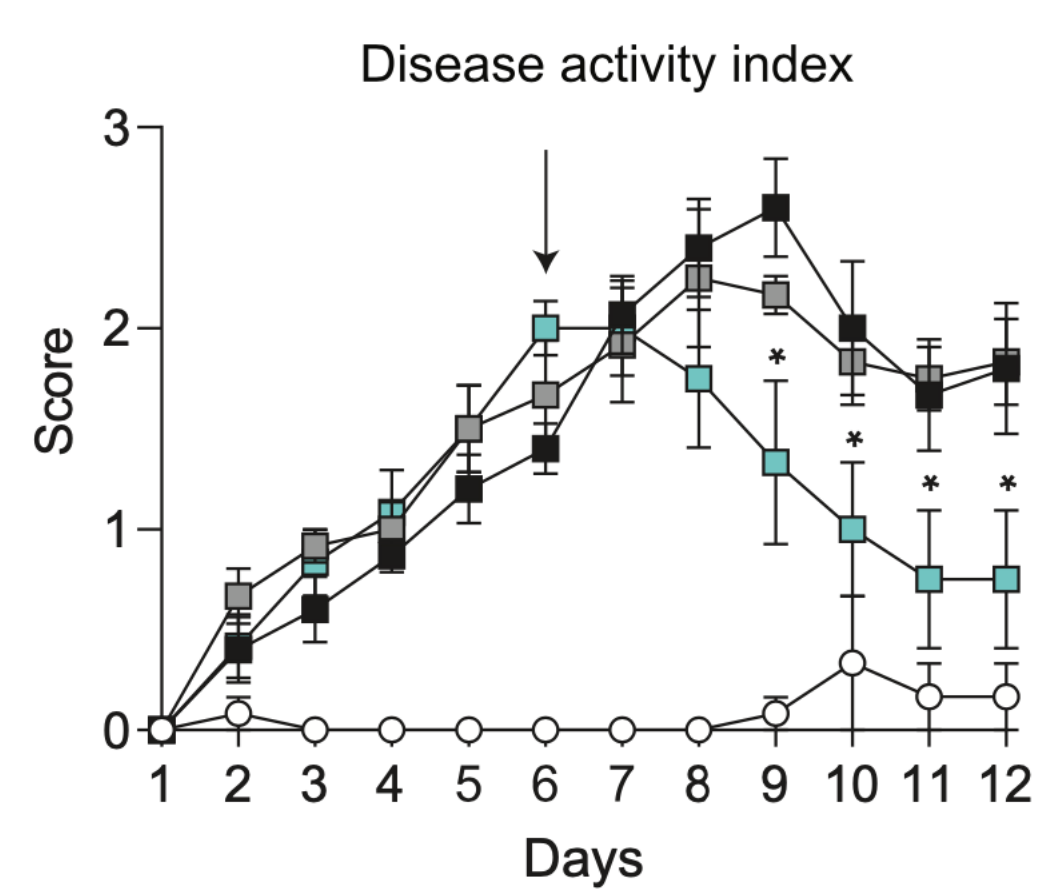


Figure 2. DAI over time (arrow indicates start of treatment) of ICI-colitis where mice receiving both anti-PD-1 and anti-CTLA-4 (ICIs) treatment were challenged by DSS

R2LC_CXCL12 induces CXCR4 signaling in Peyer's patches germinal centers, reduces surface expression of CXCR4 in small intestinal immune cells, and induces tolerogenic immune cell subsets in colon

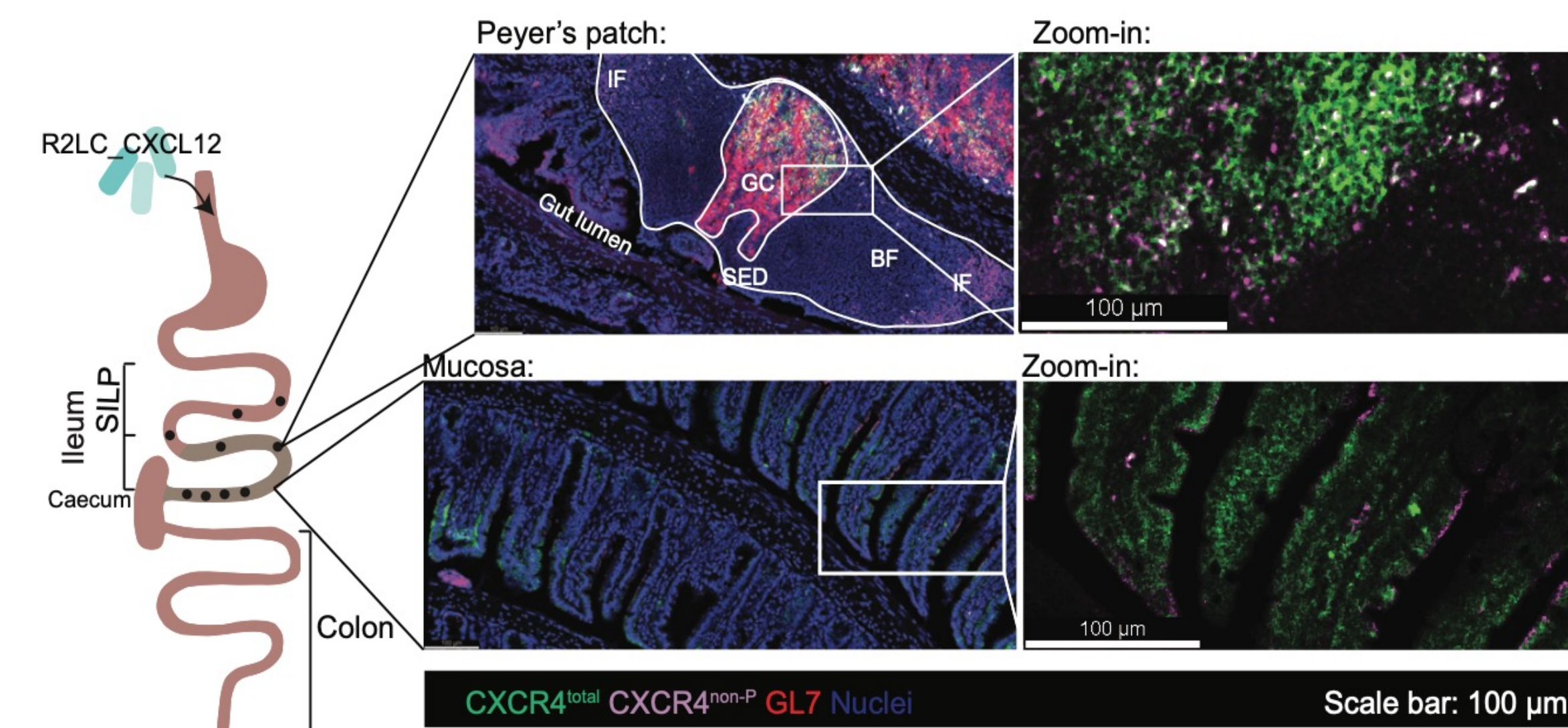


Figure 3. Representative immunofluorescent image of nuclei (blue), GL7 (red), CXCR4total (green), and CXCR4non-P (magenta) staining in the Peyer's patches (PPs) and ileal mucosa of mice with DSS induced colitis 8 h after administration of vehicle.

Product feasibility, safety and tolerability

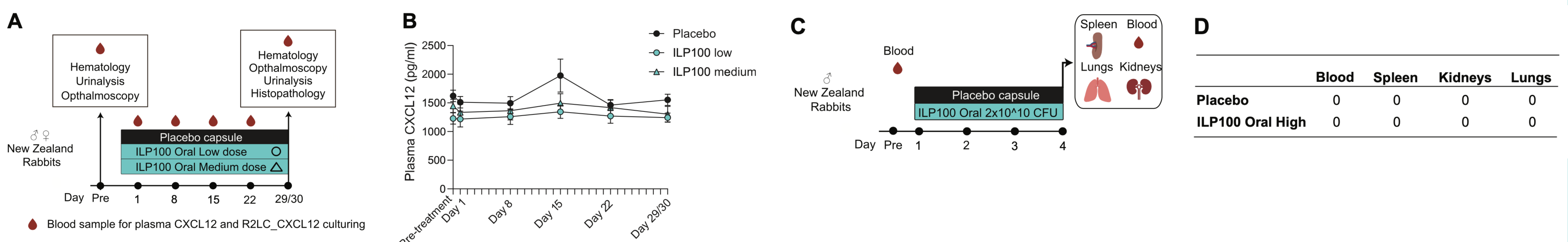


Figure 4. A: schematic overview of the experimental design for a 4 weeks multidose GLP study in New Zealand rabbits. B: plasma CXCL12 levels of placebo. C: Schematic overview of the experimental design of a GLP biodistribution study in New Zealand rabbits sampling organs under optimized conditions keeping sterility. D: number of colonies in blood, spleen, kidneys, and lungs

