

The Importance of NFkB Proteins in Dextran Sodium Sulphate Colitis

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epistem

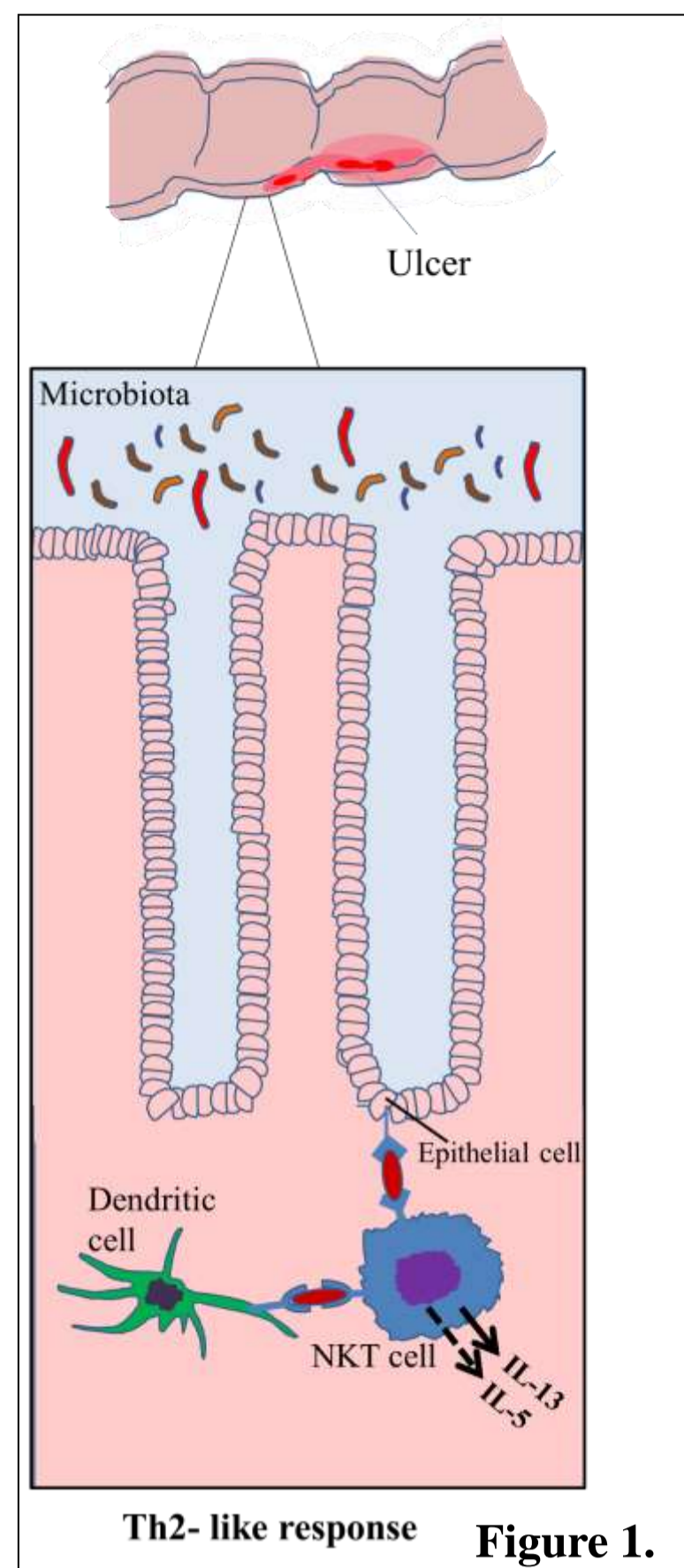
Introduction

The inflammatory bowel disease ulcerative colitis (UC) affects up to 24.5/100,000 people worldwide¹. Clinical symptoms include diarrhoea and rectal bleeding. UC is characterised histologically by Th2 like inflammation in the mucosa of the colon (Figure 1) and epithelial cell disruption.

Dextran Sodium Sulphate (DSS) is used to model UC in mice. It disrupts the mucosal barrier and causes an adverse immune response to commensal bacteria in the distal colon², causing inflammation with a Th2 cytokine profile⁴.

The NFkB family of transcription factors regulate inflammatory mediators via the classical and alternative signalling pathways. NFkB subunits NFkB1, c-Rel and RelA are involved in the classical pathway, whereas NFkB2 and RelB regulate the alternative pathway.

We have recently shown that NFkB2 null mice are protected from DSS colitis.³ However the cell population responsible for this phenotype remains to be determined.



Aim: To determine the effect of deletion of members of the NFkB family from different cell populations on DSS induced colitis

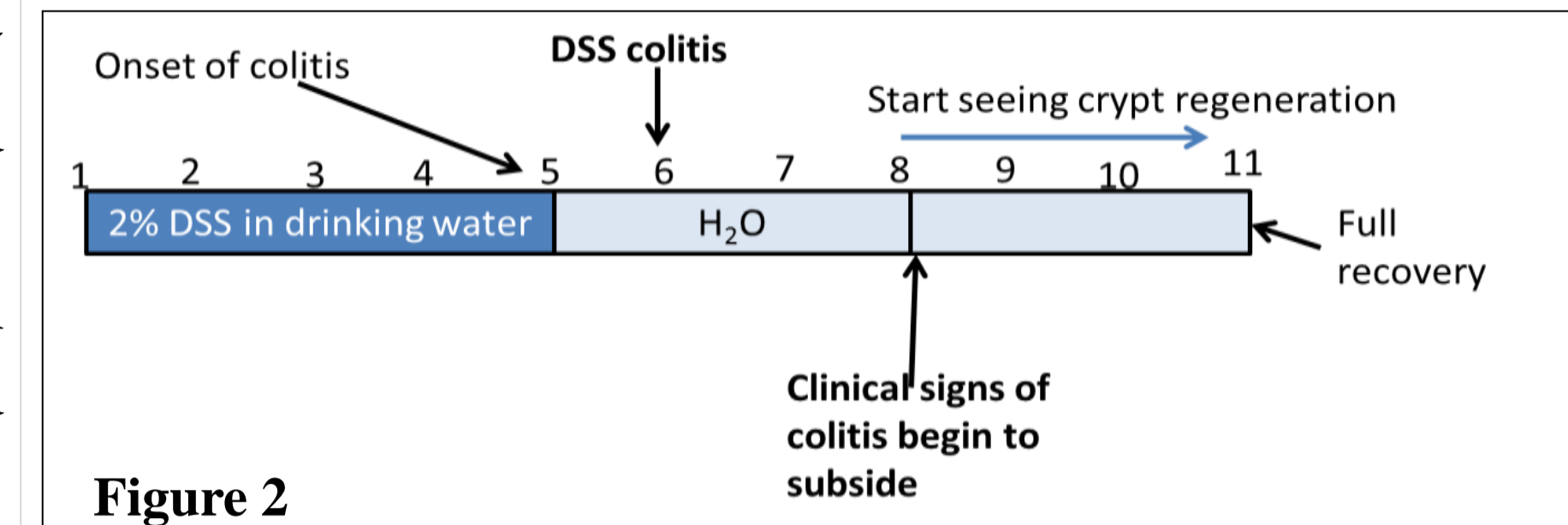
Methods

Induction of DSS colitis:

- Male C57BL/6, NFkB2 null, c-Rel null, NFkB1 null mice aged 10-12 weeks.
- 2% DSS in drinking water from day 0-5 and drinking water from days 5-11 of study.
- Control mice given drinking water throughout study.
- N=3 mice culled on day 5, 8 and 11 of study Colons taken for standard histology.
- Mouse weights taken daily throughout studies.

Bone Marrow reconstitution:

- Mice are subjected to full body irradiation- two doses of 550rad (5.5Gy) 3 hours apart.
- Animals then tail vein injected with bone marrow from donor mice and left for 6 weeks to recover.
- DSS administered to induce colitis.



Crypt survival assay method:

- Mice from DSS study with day 8 end point. 10x circumferences counted per mouse.
- Crypts in a circumference counted, and 10 widths per circumference counted.
- Mean average surviving crypt per group adjusted using following formula to adjust for crypt width:
Adjusted value = number of surviving crypts x mean crypt width control/mean crypt width treated

Results 1. Dextran sulphate sodium induces clinical signs of colitis in C57BL/6 (wild-type) mice.

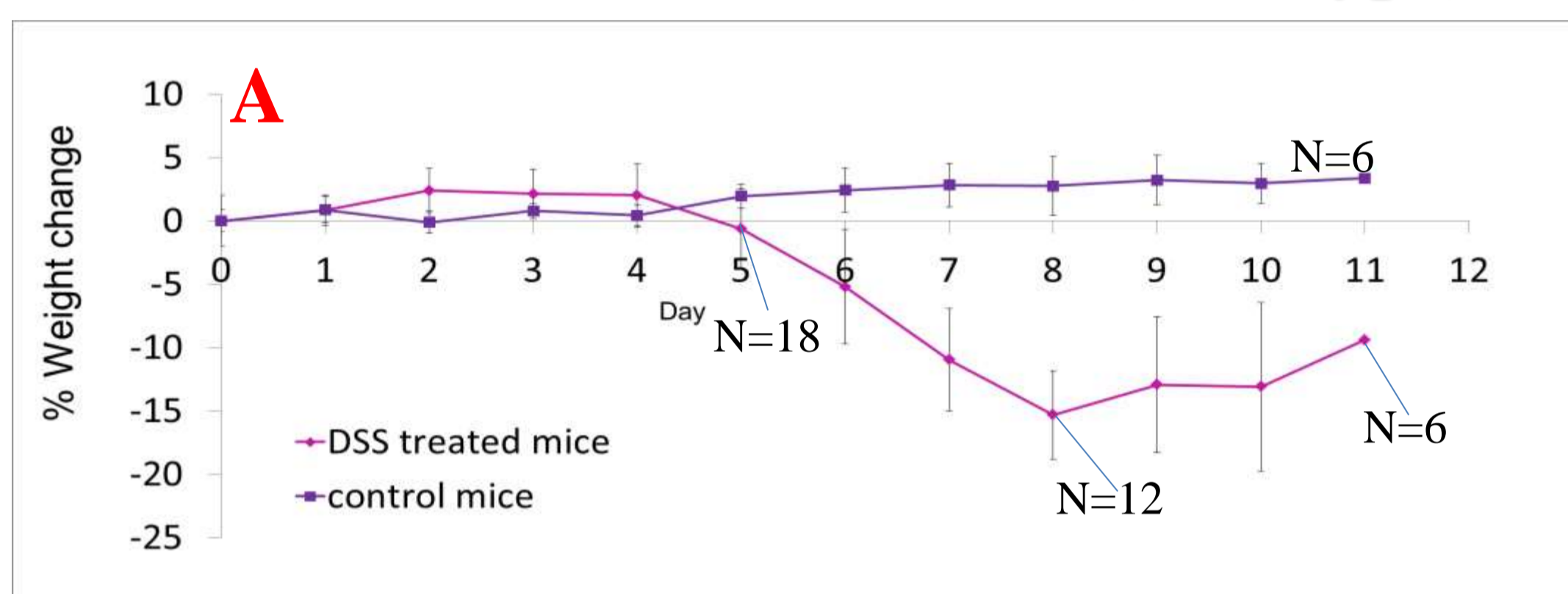
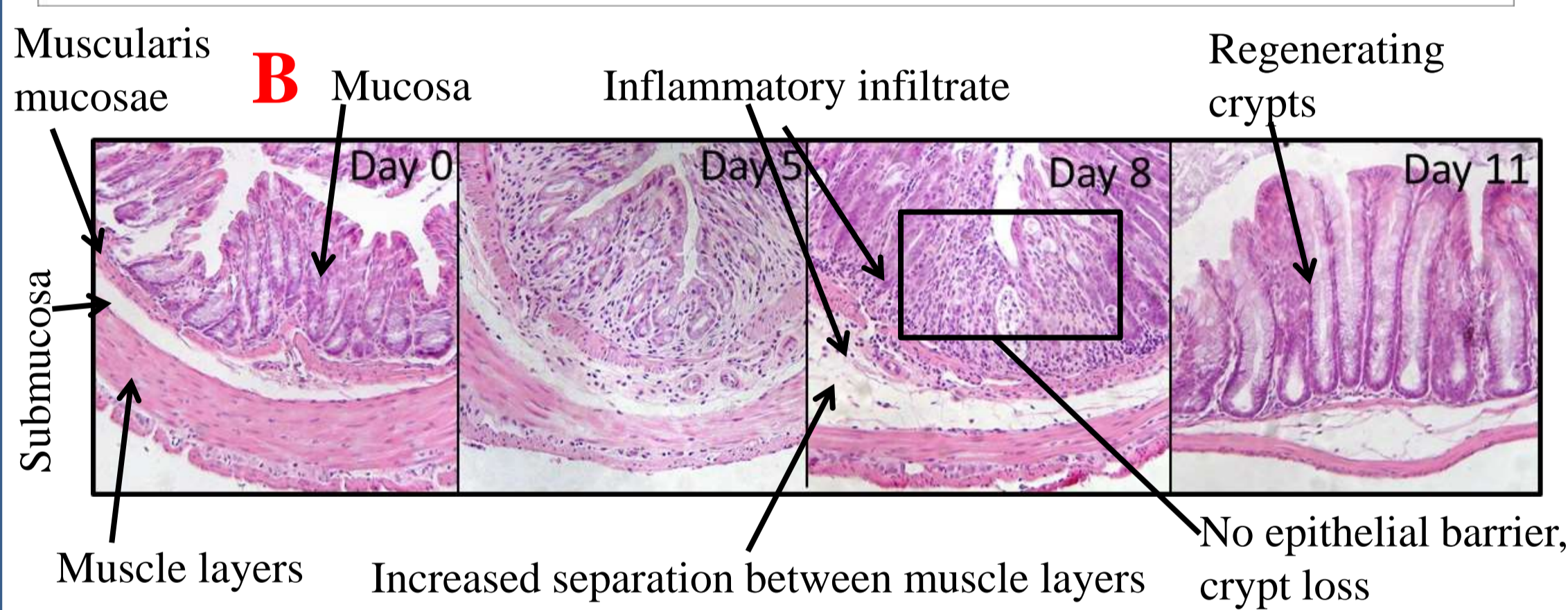


Figure 3.

- DSS treated mice exhibit weight loss (A).
- H+E stained sections of murine distal colon show histological changes in the murine colon during colitis induction and recovery (B).



Results 2. Colitis is ameliorated following deletion of alternative pathway member NFkB2.

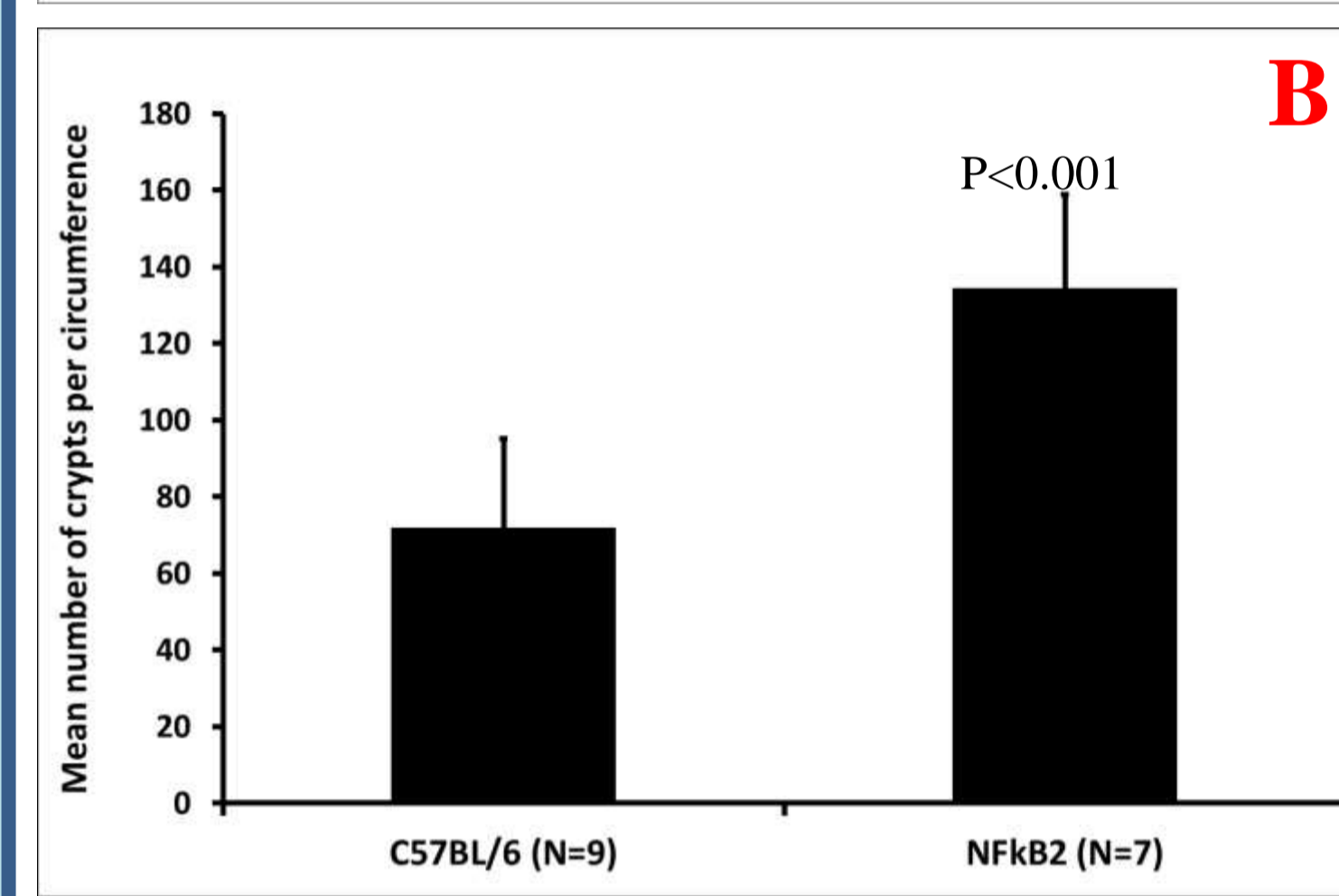
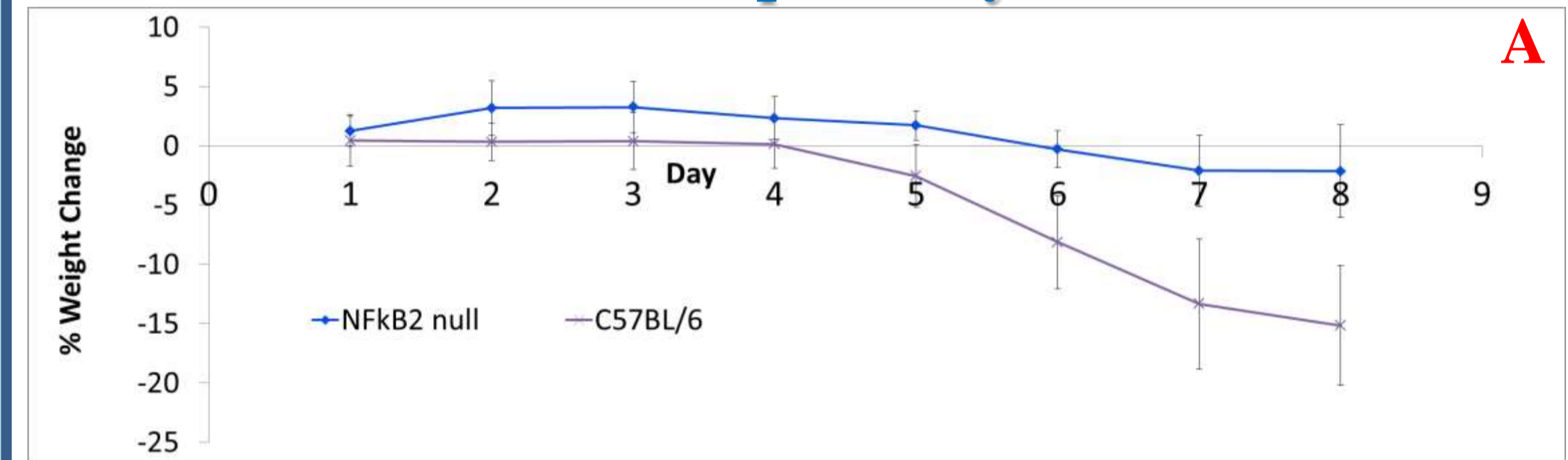


Figure 4.

- NFkB2 null mice are resistant to DSS colitis compared with C57BL/6 mice, (A) and do not get diarrhoea or rectal bleeding.
- NFkB2 null mice had significantly more surviving crypts than C57BL/6 wild-type mice (B).

Results 3. NFkB1 null and c-Rel null mice are susceptible to DSS induced colitis.

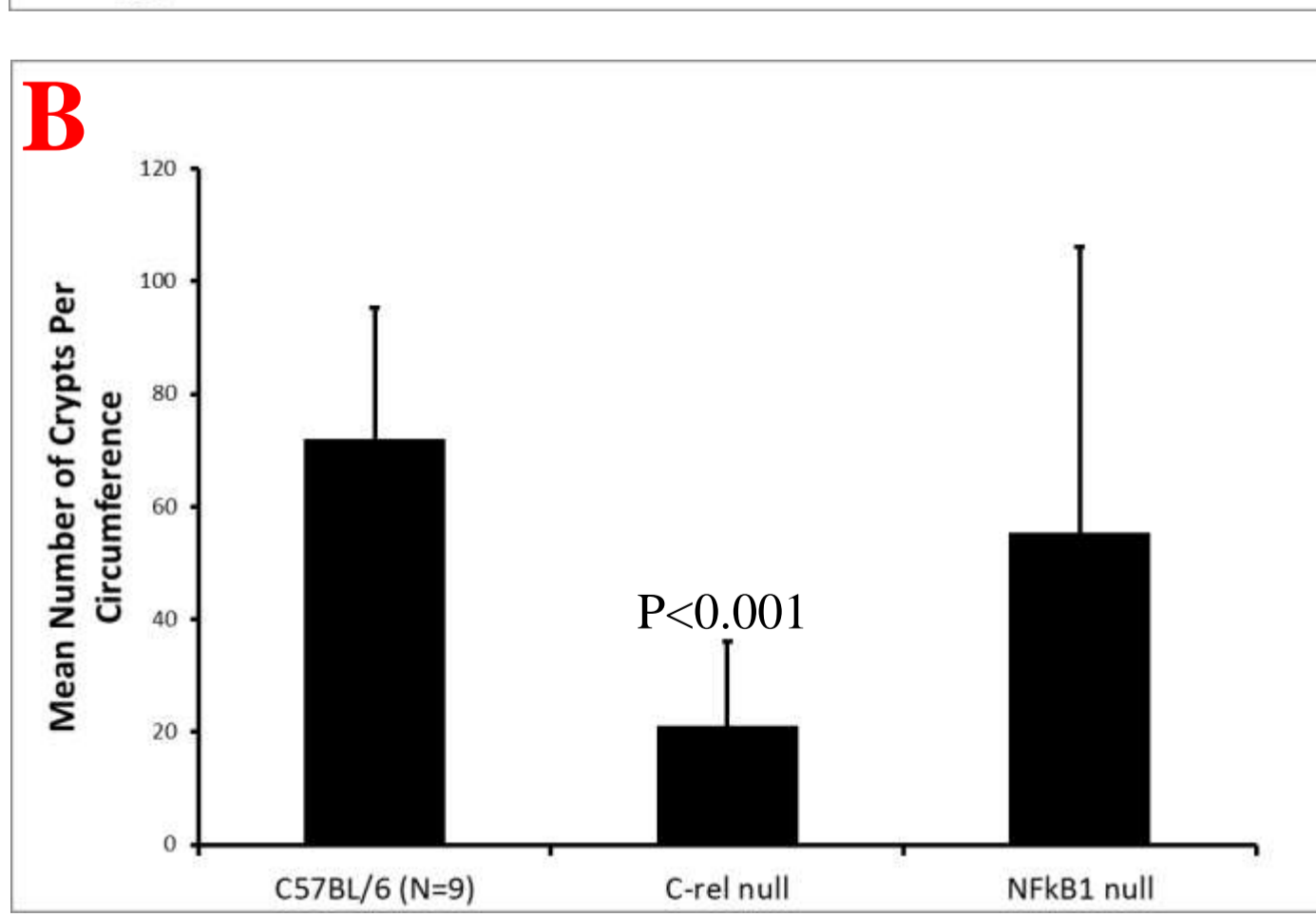
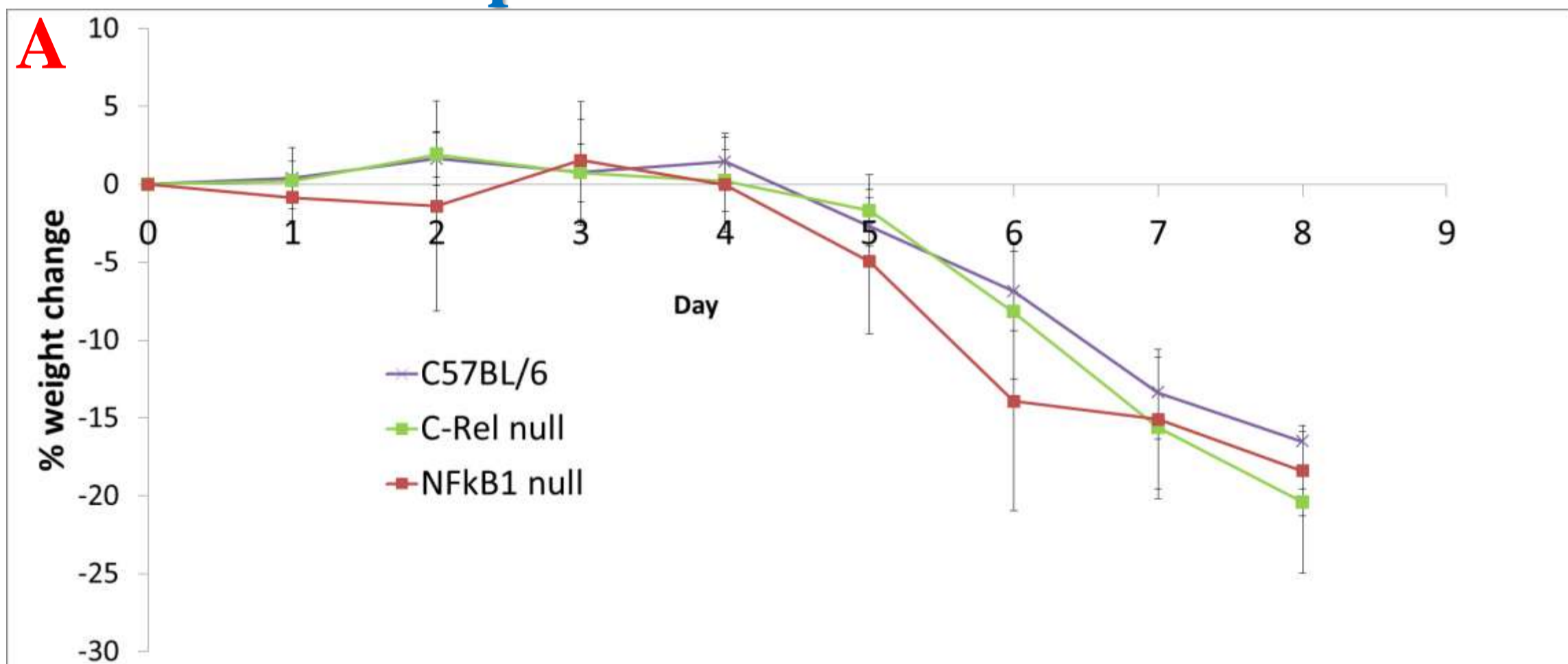


Figure 5.

- C-Rel null and NFkB1 null mice are slightly more sensitive to colitis induction than C57BL/6, although this did not reach significance in weight loss (A) for both genotypes and crypt survival assay for NFkB1 null mice (B).

Results 4. Epithelial cell specific deletion of NFkB2 reduces colitis severity.

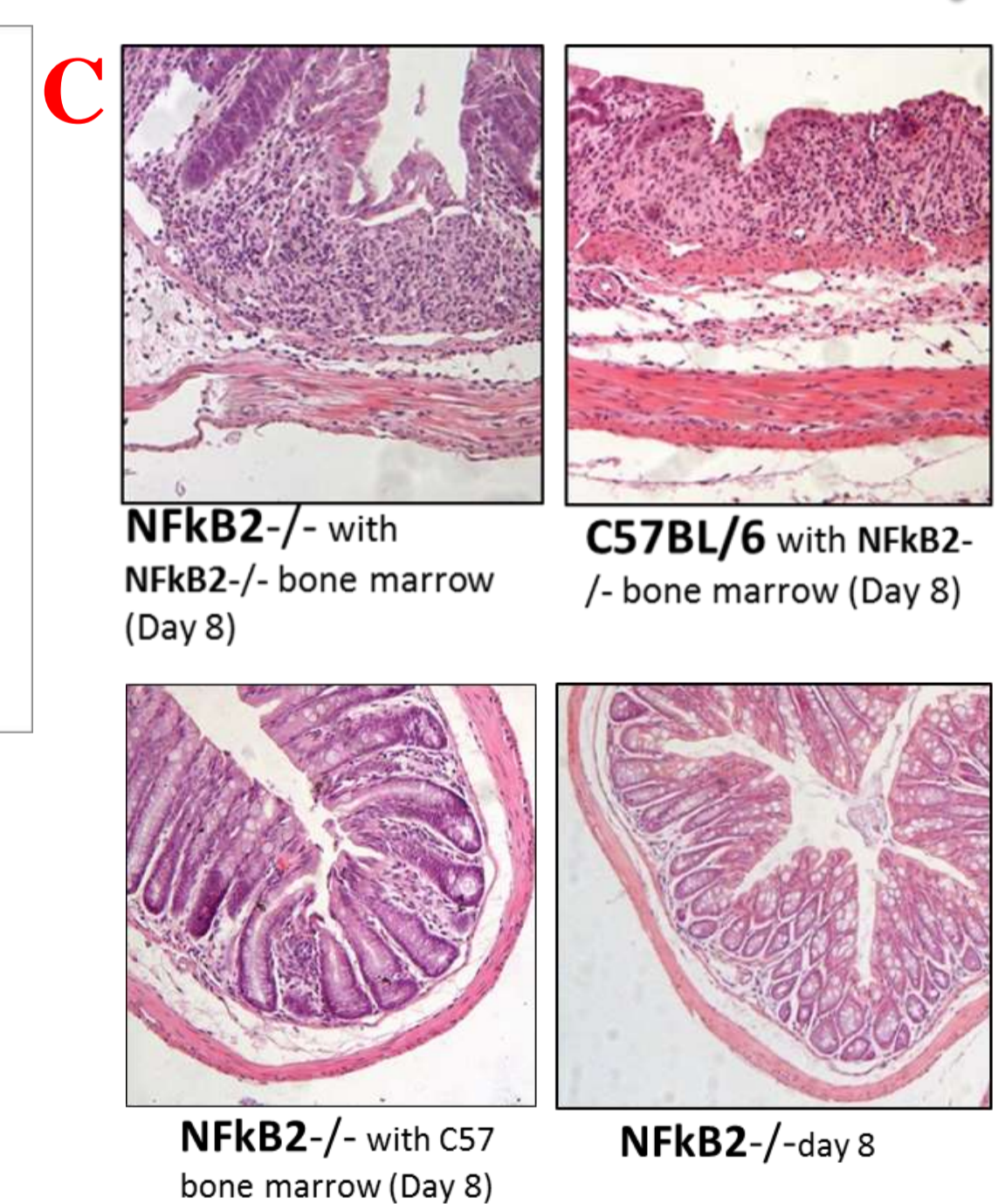
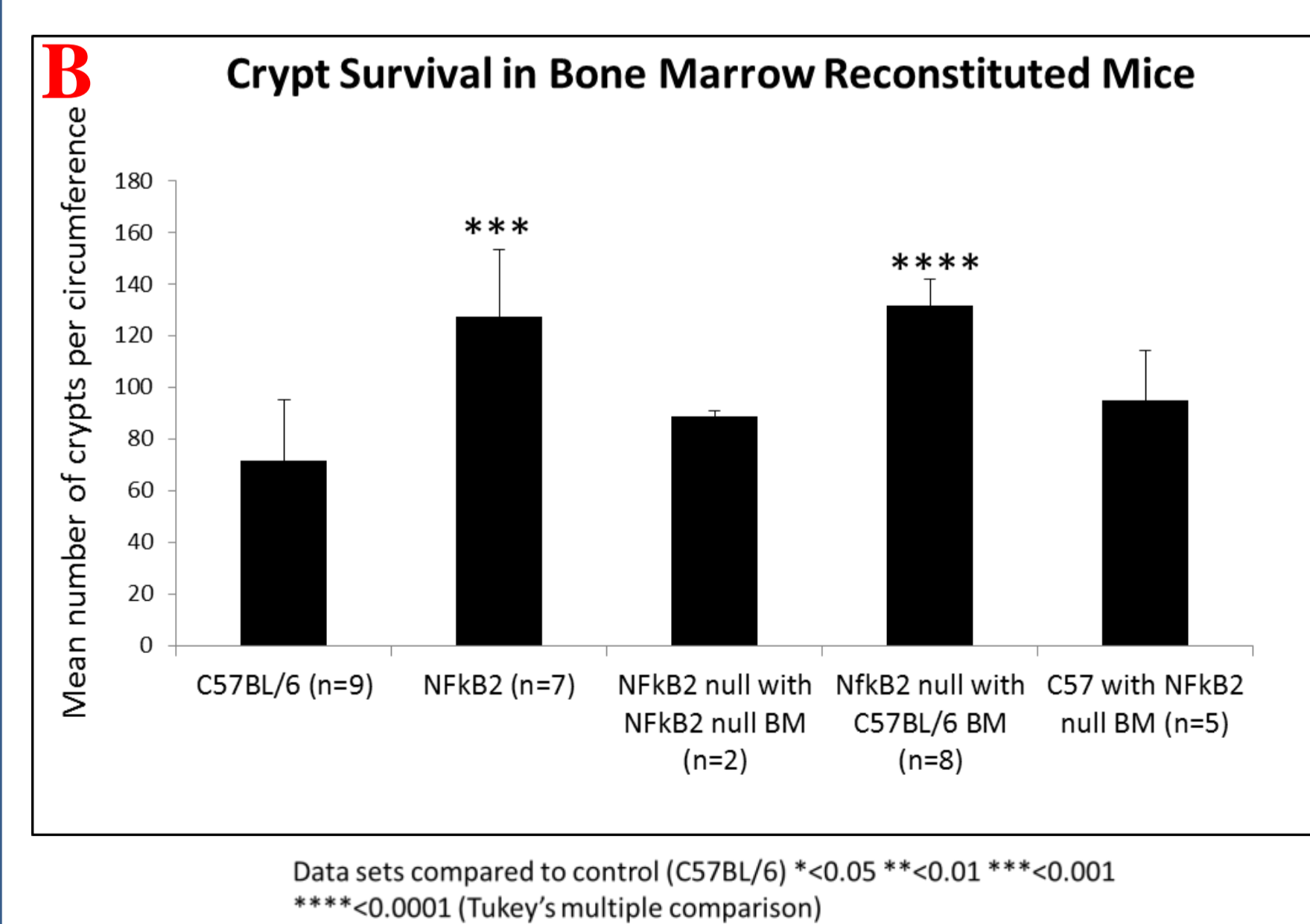
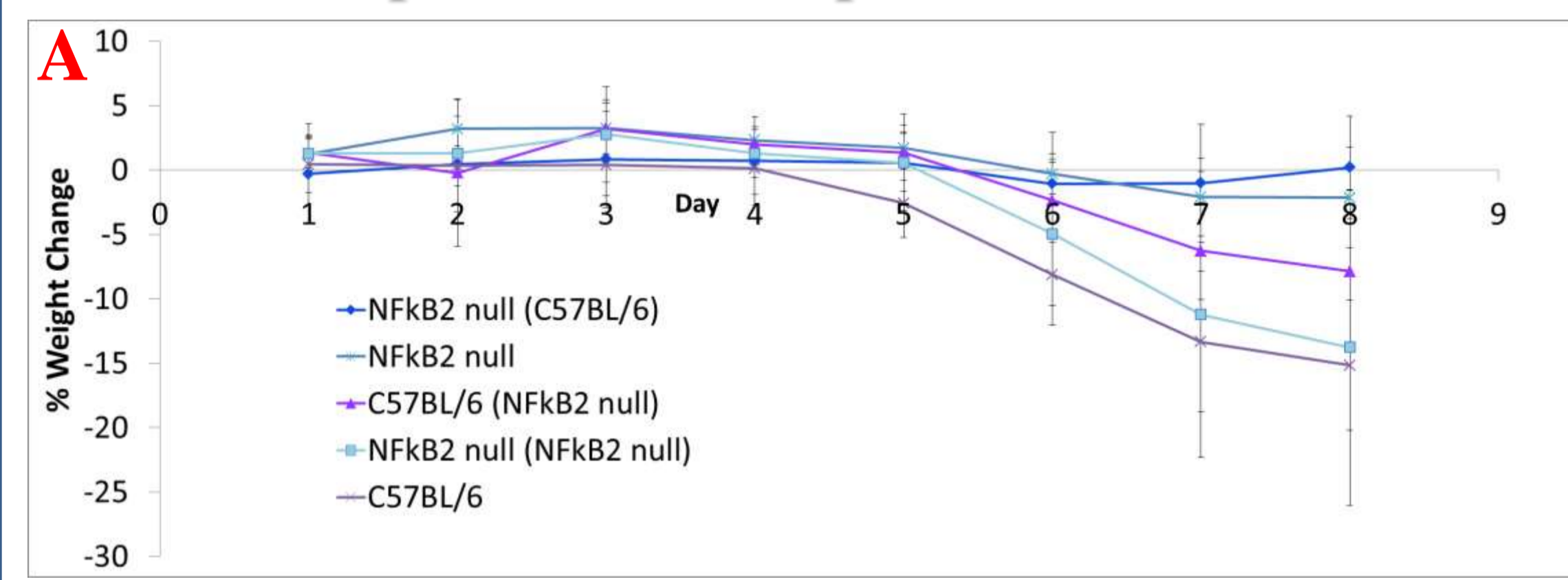


Figure 6

- Results indicate that the epithelial compartment that plays a role in the sensitivity to induction of DSS colitis.
- Bone marrow reconstitution was confirmed by standard PCR (data not shown).
- Bone marrow reconstituted mice shown in A as 'genotype (bone marrow genotype).'

Conclusions

NFkB2 null mice are protected from colitis, but c-rel and NFkB1 null mice are sensitive to DSS colitis. The effect of NFkB subunit knockouts on DSS colitis indicates that the classical and alternative pathways may have different roles in colitis induction, and that the alternative pathway (to which NFkB2 belongs) may have a significant role in colitis progression.

Using bone marrow reconstitution we have shown that the genotype of the bone marrow derived cells contributes less to DSS colitis induction than the genotype of the epithelium.

Further work: To determine the cellular downstream mechanisms by which NFkB2 signalling contributes to colitis pathogenesis.

References

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