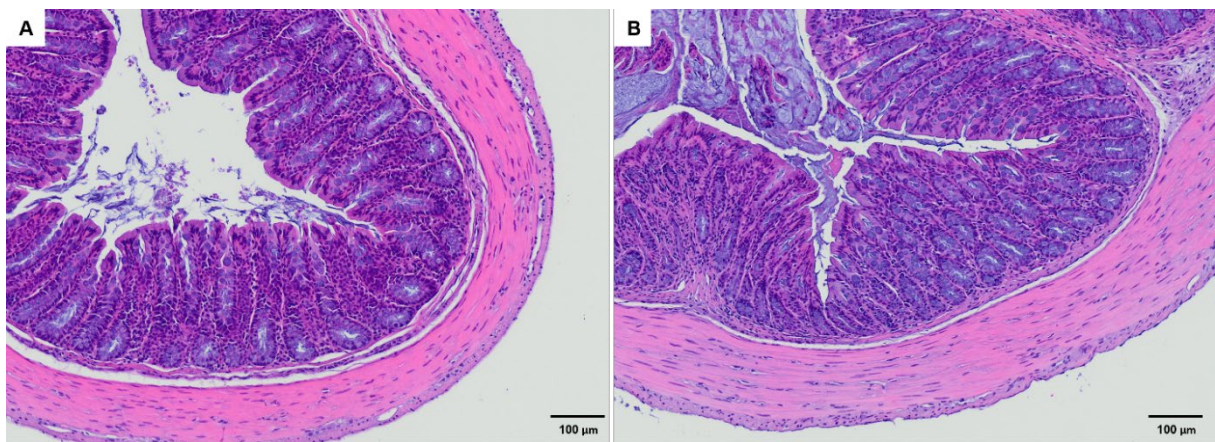


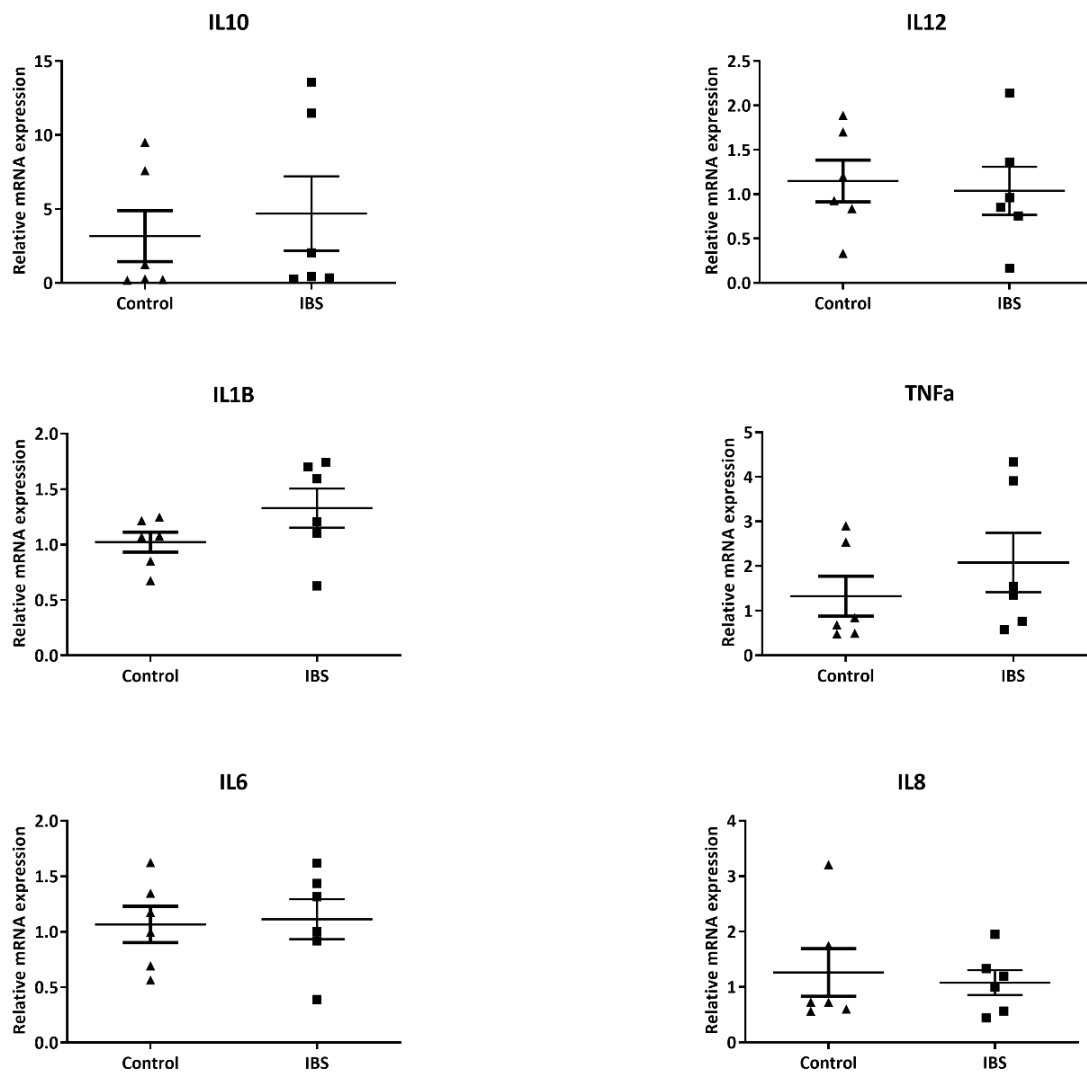
For microscopic evaluation of inflammation in the mouse colon, a colonic segment was fixed in 4% formaldehyde for 24h and embedded in paraffin for hematoxylin & eosin staining.

H&E stainings from control and neonatal acetic acid-induced IBS mice (n=6/group) were microscopically scored using a previously published scoring system (Heylen et al., 2013; Van Spaendonk et al., 2021). Briefly, the following parameters were scored: degree of inflammatory infiltrates (0-3), presence of goblet cells (0-1), changes in crypt architecture (0-3), presence of crypt abscesses (0-1), presence of mucosal erosion and/or ulceration (0-1) and the number of layers involved in inflammation from mucosal towards transmural involvement (0-3), resulting in a total score ranging between 0 and 13. The samples were scored in a blinded way and all parameters showed a score of 0 both in control mice and neonatal acetic acid mice. Representative images from an H&E staining of the colon of a control and IBS mouse are shown in **Figure 1**.



**Figure 1.** Representative image from an H&E staining of the colon of a control (A) and a neonatal acetic acid-induced IBS mouse (B) in their adulthood.

Next to H&E stainings, mRNA cytokine levels (IL-10, IL-12, IL1B, TNF $\alpha$ , IL6, IL8,) were quantified by RT-qPCR using a previously published method (Plaek et al., 2020) in colon samples from control vs acetic acid-induced IBS mice. The sequences of the primers as well as the housekeeping genes used (*Rpl4* and *Rps29*) are shown in **Table 1**. No significant differences were found between control and IBS mice (**Figure 2**).



**Figure 2.** mRNA cytokine levels (IL-10, IL-12, IL-1B, TNFa, IL6, IL8) in colon samples from control vs acetic acid-induced IBS mice. Unpaired T-test (IL-12, IL-1B, IL-6) or Mann-Whitney U test (IL-10, TNFa, IL8). No significant differences were found between control and IBS mice.

**Table 1.** SYBR Green primers used for RT-qPCR analysis of mouse colonic samples

Primer name	Sequence 5'-3'
m_RPL4_FW	CCGTCCCCTCATATCGGTGTA
m_RPL4_REV	GCATAGGGCTGTCTGTTGTTTT
m_RPS29_FW	GTCTGATCCGCAAATACGGG
m_RPS29_REV	AGCCTATGTCCTTCGCGTACT
IL-10_m_FW	GCTCTTACTGACTGGCATGAG
IL-10_m_REV	CGCAGCTCAGGAGCATGTG
IL12_m_FW	CAATCACGCTACCTCCTCTTT
IL12_m_REV	CAGCAGTGCAGGAATAATGTTTC
IL1beta_m_FW	GAAATGCCACCTTTTGACAGTG
IL1beta_m_REV	TGGATGCTCTCATCAGGACAG
TNFalfa_m_FW	CAGGCGGTGCCTATGTCTC
TNFalfa_m_REV	CGATCACCCGAAGTTCAGTAG
IL-6_m_FW	ACACATGTTCTCTGGGAAATCGTGG
IL-6_m_REV	TCTGCAAGTGCATCATCGTTGTCA
IL8_m_FW	TCGAGACCATTACTGCAACAG
IL8_m_REV	CATTGCCGGTGGAAATTCCTT

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