

Xiaoyu Wang<sup>1</sup>, Shih-Yen Weng<sup>1</sup>, Tao chen<sup>1</sup>, Olena Molokanova<sup>1</sup>, David Fraser<sup>2</sup>, Detlef Schuppan<sup>1,3</sup>.

<sup>1</sup> Institute of Translational Immunology and Research Center for Immune Therapy, University Medical Center, Mainz, Germany, <sup>2</sup> Jecurion Therapeutics, Oslo, Norway <sup>3</sup> Division of Gastroenterology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, USA

## BACKGROUND & AIMS

Glucagon-like peptide-1 (GLP-1) improves insulin sensitivity via enhanced glucose-dependent insulin secretion, inhibition of glucagon release, and delayed gastric emptying following its release into the circulation from the gut. We aimed to explore the utility of a long-acting GLP-1 receptor agonist (Bydureon, BY) on liver steatosis, inflammation and fibrosis in an optimized choline deficient, amino acid defined (CDAA), 0.2% cholesterol high fat diet model of NASH, focusing on immune cells and especially macrophages, considered a cell population with high relevance in the pathogenesis of NASH.

## MATERIALS & METHODS

Male C57BL/6 mice were fed the CDAA (CSAA=choline sufficient, control) diet for 12 weeks, BY was administered twice weekly by subcutaneous injection of 0.4 (BY-L) or 2 (BY-H) mg/kg to C57BL/6 mice fed the CDAA diet for 6 weeks.

Hepatic fibrosis was assessed by morphometric analysis of Sirius red stained collagen and measurement of hydroxyproline content. Fibrosis, inflammation and metabolism related transcripts were measured by quantitative real-time polymerase chain reaction (qPCR). Select inflammatory and fibrosis markers were quantified by immunohistochemistry. Ex vivo analysis of hepatic inflammatory cells was performed by FACS.

## RESULTS

Fig.1: Bydureon attenuated insulin resistance in injured livers of CDAA diet-fed mice

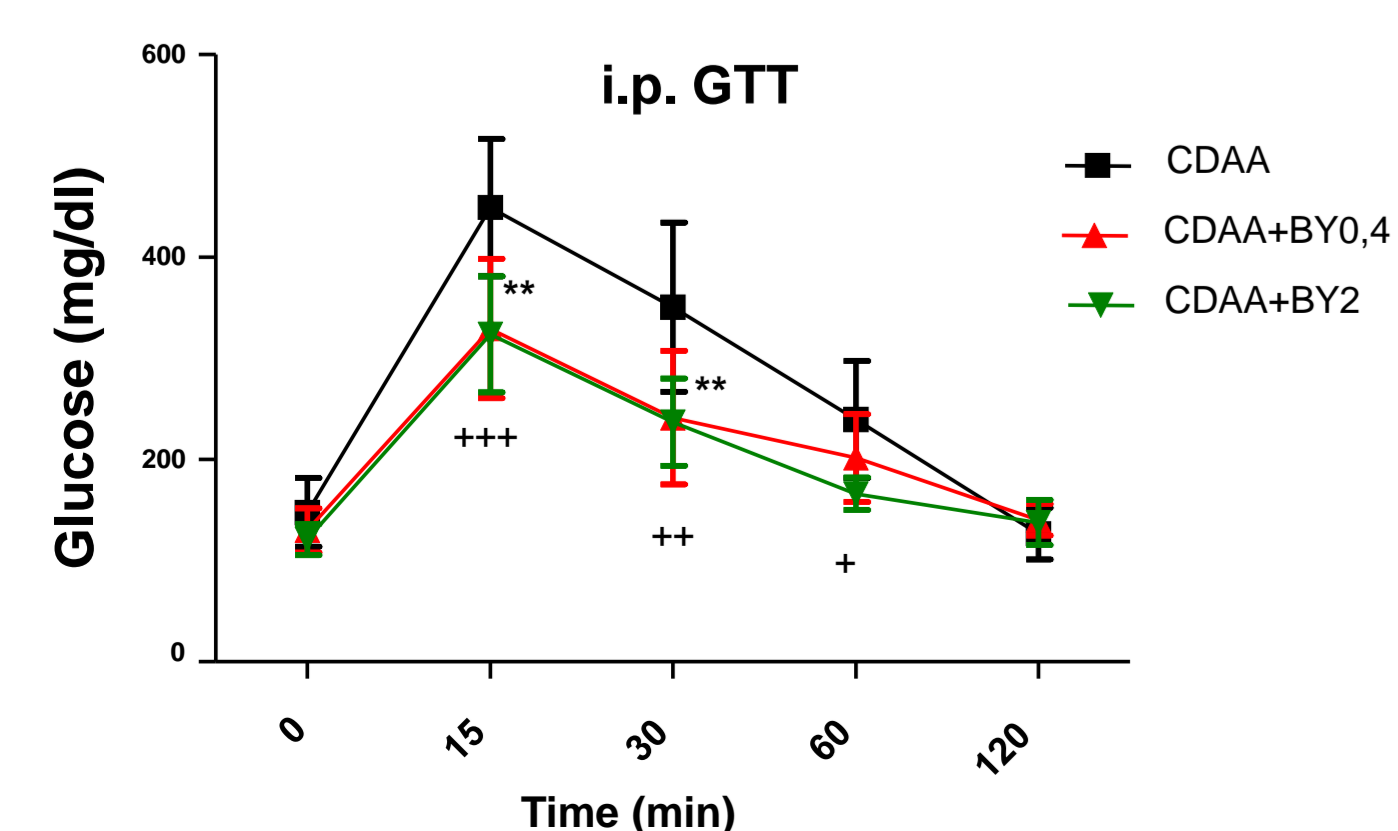


Figure 2: Food intake, Body, liver and epididymal weights, and serum biochemical parameters of CDAA diet-fed mice

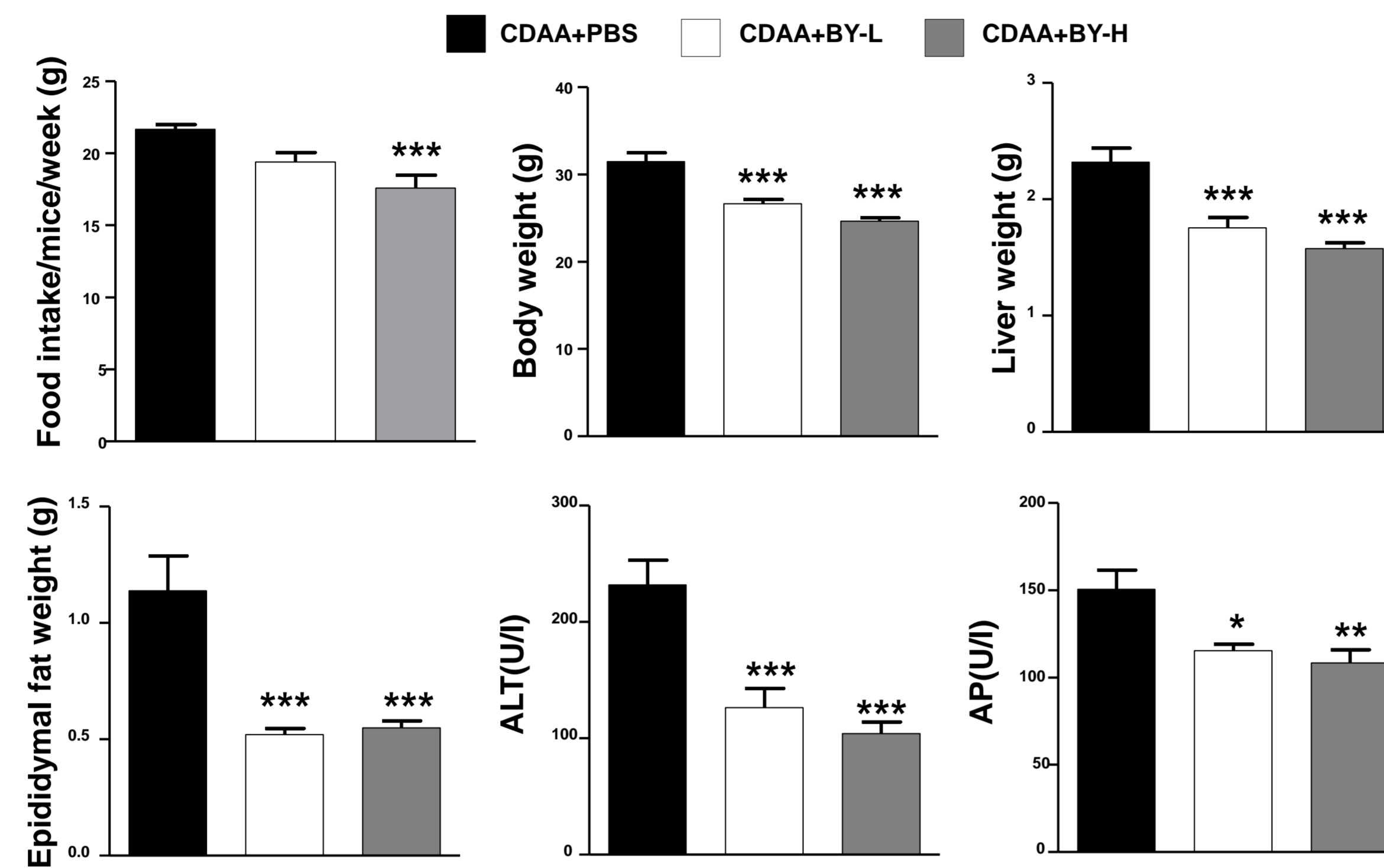


Fig. 3: Bydureon treatment reduces Hyp content in the CDAA diet mouse model

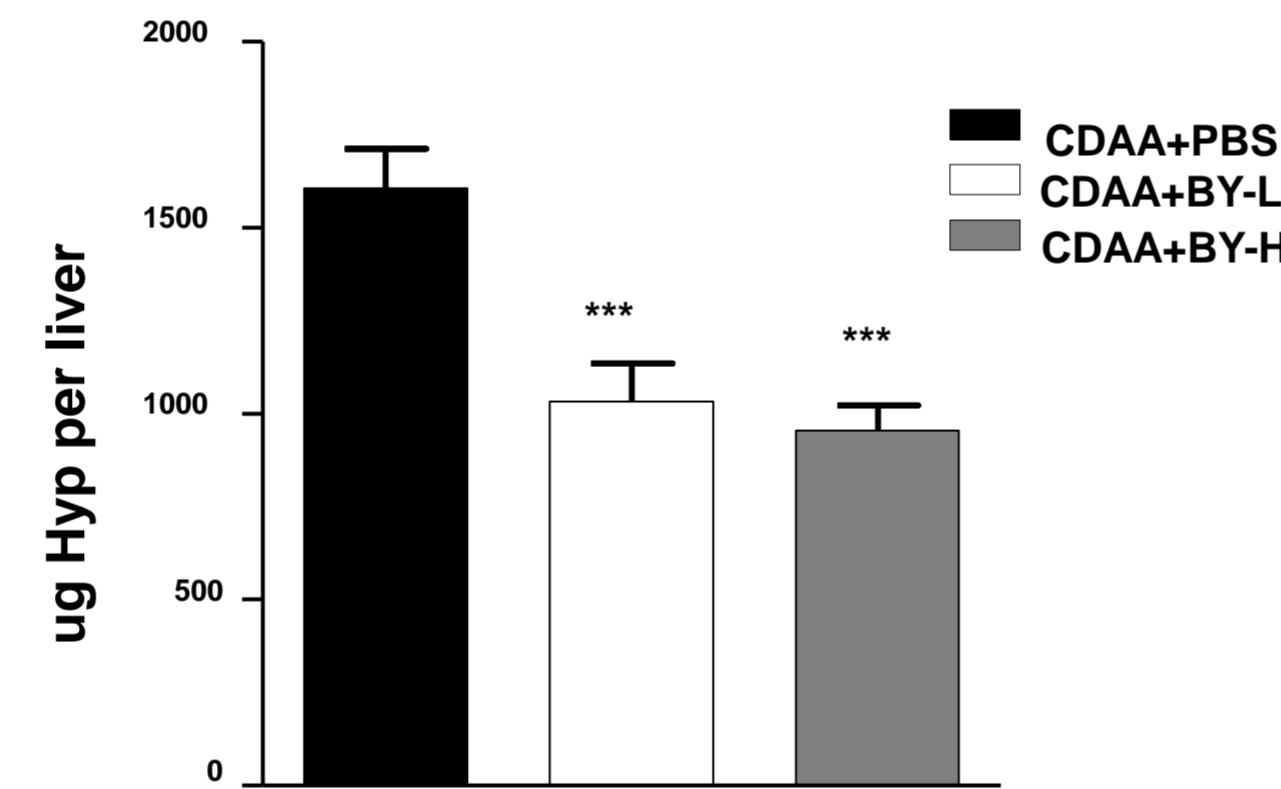
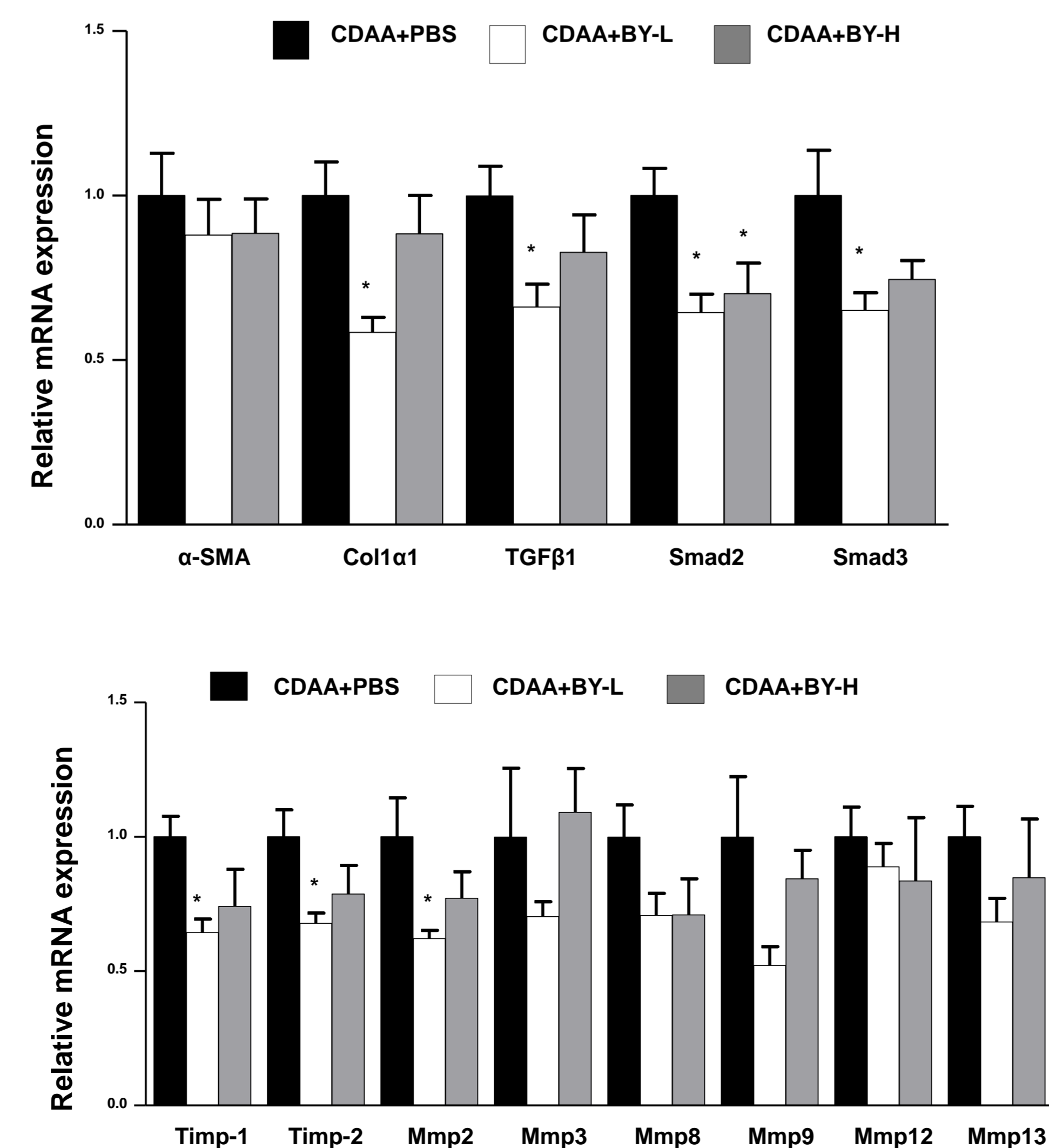


Fig.4: Bydureon treatment reduces fibrosis related transcript levels in the CDAA diet mouse model



## RESULTS

Fig. 5: Bydureon treatment suppress hepatic fibrosis in the CDAA diet mouse model

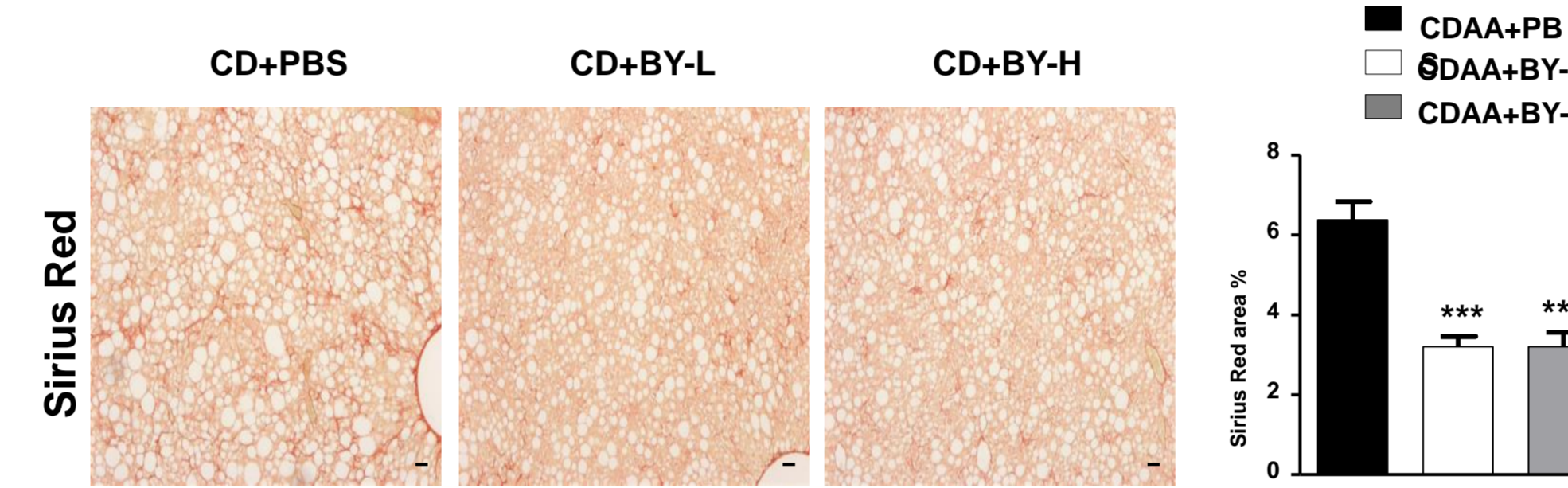


Fig. 6: Bydureon treatment reduces inflammation related transcript levels in the CDAA diet mouse model

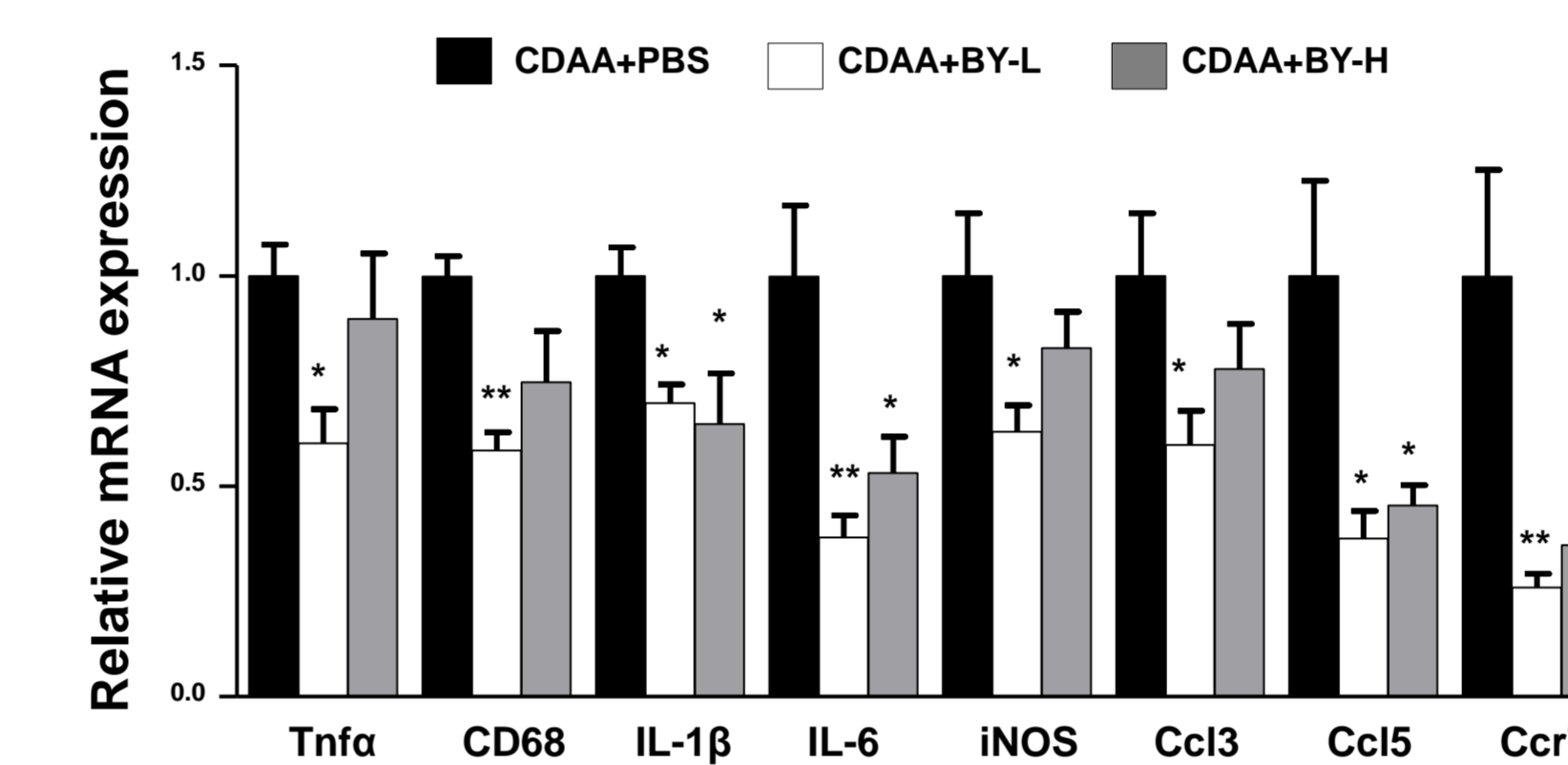


Fig.7: Fluorescence-activated cell sorting analysis of liver leukocytes in PBS and BY-treated mice.

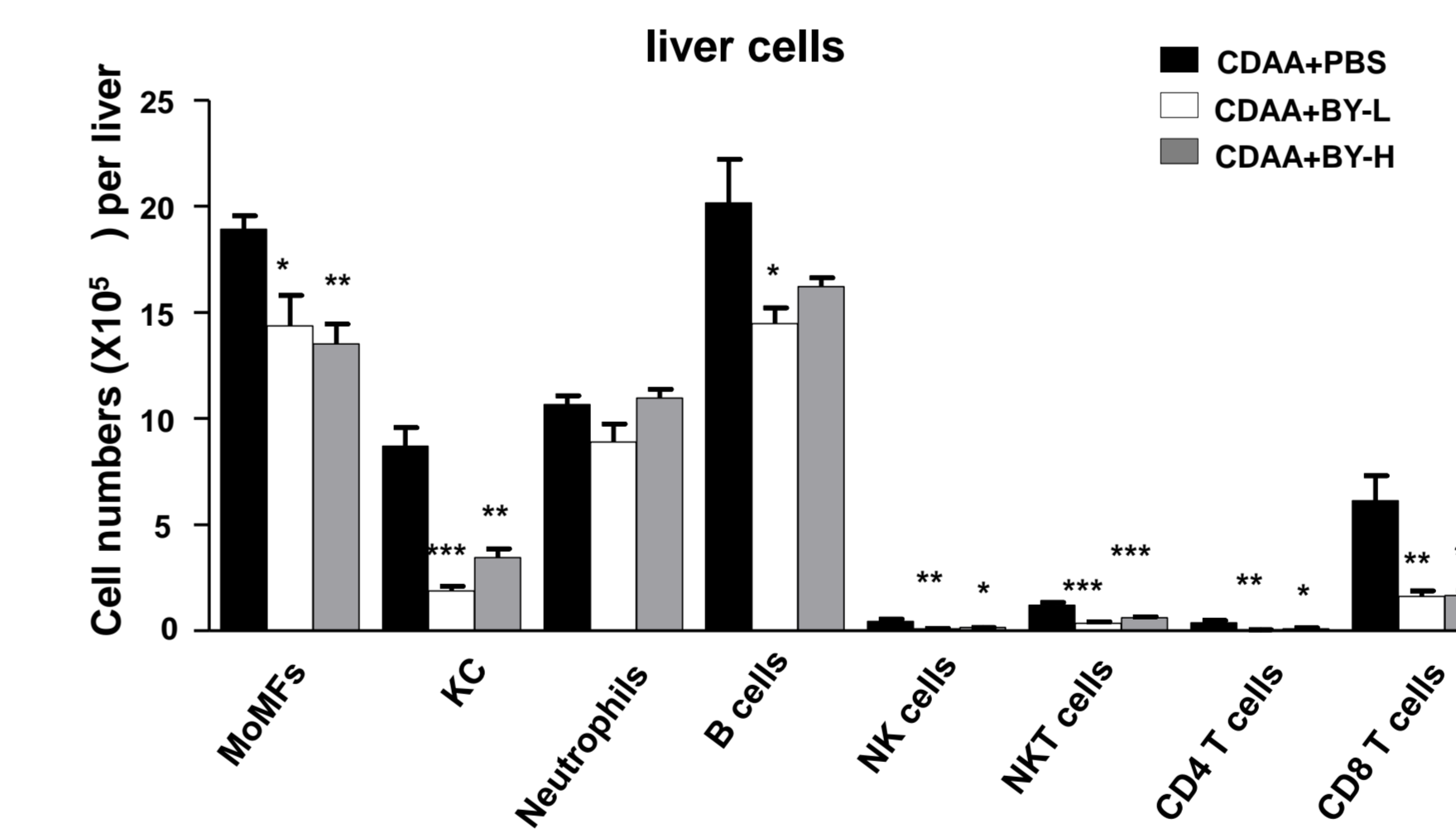
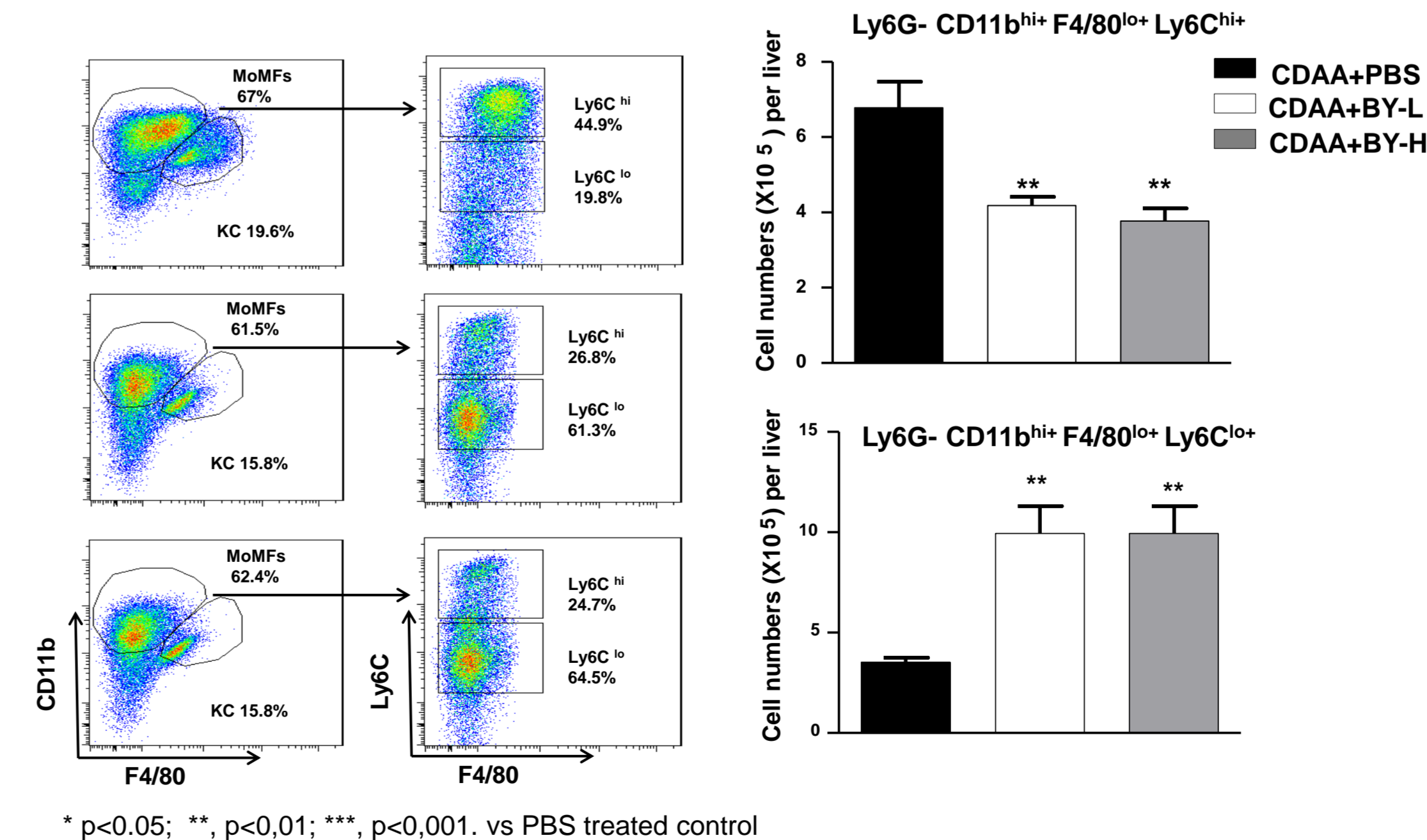
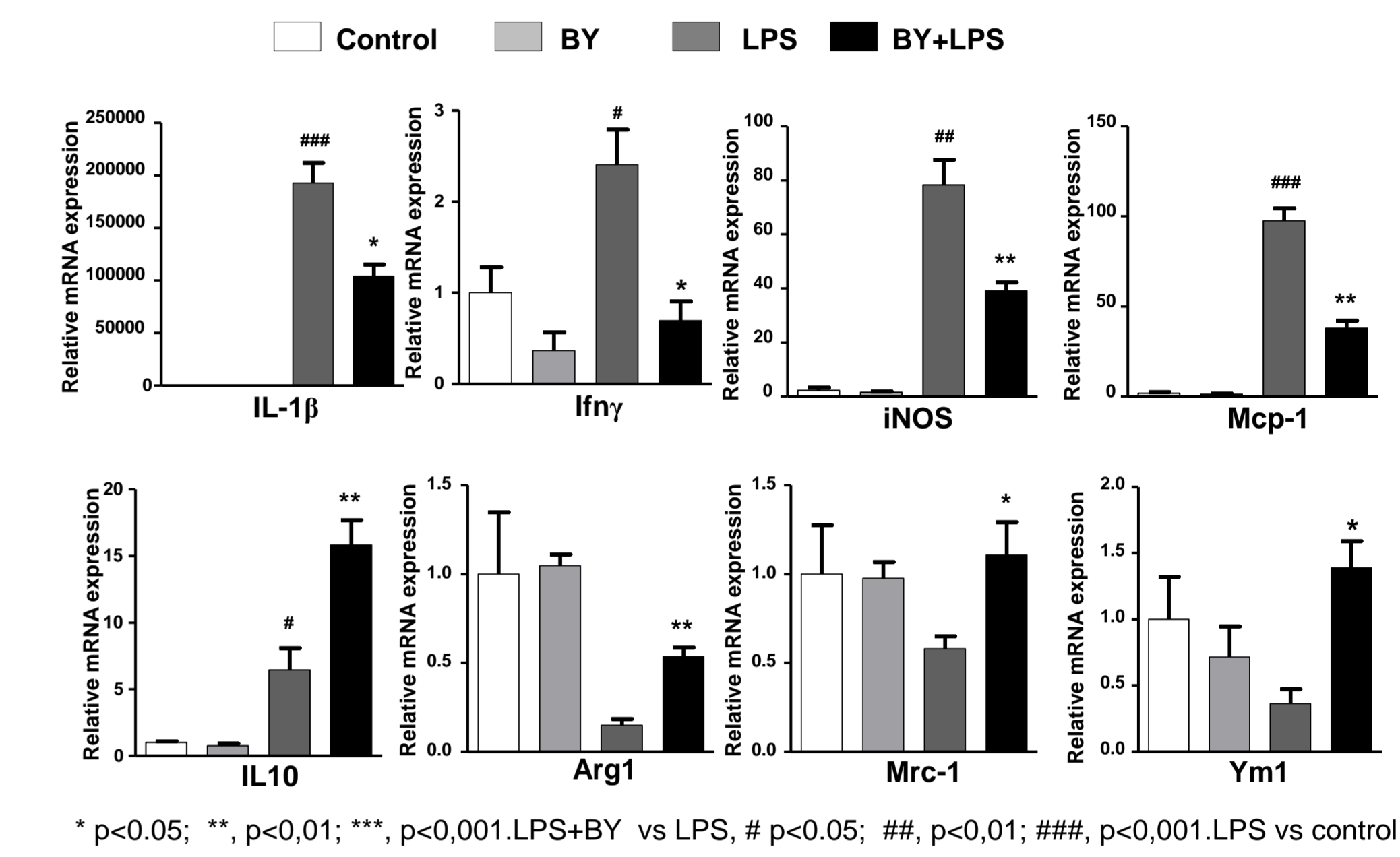


Fig. 8: Representative flow cytometry analysis of monocyte-derived macrophages (MoMF).



\* p<0.05; \*\* p<0.01; \*\*\* p<0.001. vs PBS treated control

Fig. 9: *In vitro* treatment, Bydureon suppress LPS-stimulated M1 macrophage markers, increased M2 macrophage related markers.



\* p<0.05; \*\* p<0.01; \*\*\* p<0.001. LPS+BY vs LPS, # p<0.05; ## p<0.01; ### p<0.001. LPS vs control

## RESULTS

All mice gained weight. At the lowest dose, BY significantly maximally decreased body and liver weight, ALT, AST, fasting glucose and insulin resistance, liver collagen accumulation, fibrosis related (Col1α1, α-SMA, TIMP-1, TGFβ1), several MMP) and inflammation related transcripts levels (Tnfa, CD68, IL-1β, IL-6, CCL3).

Flow cytometry analysis of liver mononuclear cells showed a reduction of Kupffer cells and monocyte derived macrophages, natural killer T cells, helper and cytotoxic T cells in BY treated CDAA diet fed animals. *In vitro* BY polarized LPS-stimulated macrophages towards M2, increasing their IL-10, Mrc1, Arg1 and Ym1 expression, and decreasing pro-inflammatory cytokines and chemokines.

## CONCLUSIONS

The optimized CDAA model reflects all features of NASH. Direct GLP-1 receptor agonists improve all these pathologies, including fibrosis. Apart from their antidiabetic and weight lowering activities, a key mechanism is their suppression of pro-inflammatory M1, and promotion of anti-inflammatory (M2) macrophage populations that may also inhibit fibrogenesis. Therefore, further clinical evaluation of the utility of GLP-1R agonists for the treatment of patients with NASH and liver fibrosis is warranted.