GDF11 in gastrointestinal disorders

Weronika Machelak, Marta Zielińska

Department of Biochemistry, Faculty of Medicine, Medical University of Lodz, MOLecoLAB Mazowiecka 5, 92-215 Lodz, Poland

International Doctoral School
Medical University of Lodz

INTRODUCTION

Growth differentiation factor 11 (GDF11) is a novel member of TGF-β superfamily. Its role is confirmed in embryogenesis, regeneration, rejuvenation, and aging. Recent data show that disturbances in GDF11 activity may affect development of cardiovascular diseases, diabetes mellitus, cancer, psoriasis, and ischemic injuries. It was found that GDF11 is involved in inflammatory response as it inhibits the release of pro-inflammatory cytokines and NLRP3 inflammasome activation. GDF11 has clinicopathological significance in colorectal cancer and is proposed as a possible prognostic factor among these patients due to its increased expression. Despite that, GDF11 remains controversial in context of liver injury due to its pro-fibrotic effect and worsening the hepatitis.

Male C57BL/6 mice were obtained from the Animal Facility of the University of Lodz, Lodz, Poland. All procedures on animals were approved by the Local Ethical Committee for Animal Experiments (38/ŁB/212/2021). Animals were maintained under a 12 hours light/dark cycle and a constant temperature (22°C) with free access to chow pellets, and tap water.

AIM

Assessment of the activity of GDF11 in the course of colorectal cancer

**Validation of the mouse model of acute hepatitis**

METHODS

Azoxymethane (AOM) at the dose of 10 mg/kg injected intraperitoneally and DSS (Dextran Sodium Sulfate) were used to induce colitis-associated colorectal cancer. Mice were sacrificed after 7 (initiation of carcinogenesis) and 11 weeks. RNA and protein were isolated from the colonic samples. The expression of GDF11 at mRNA (real-time RT-PCR) and protein (western blot) level were assessed.

D-galactosamine (GalN) was administrated at the dose of 700 µg/kg *i.p.*, 30 minutes before TNFα *i.p.* injection (0.1 µg or 0.3 µg). After 6 hours animals were sacrificed. Blood and liver samples were collected for histology, RNA and protein isolation, MPO activity measurement. The expression of *Gdf11, Tnfα, Il-6, Bax, Nos2* at mRNA level was assessed by real-time RT-PCR.

DATA ANALYSIS

ΔCt method was applied for calculation of PCR results. Normalization to housekeeping protein was applied for determination of protein relative expression. Data show mean ± Standard Deviation (SD). Statistical analysis was performed using GraphPad Software.
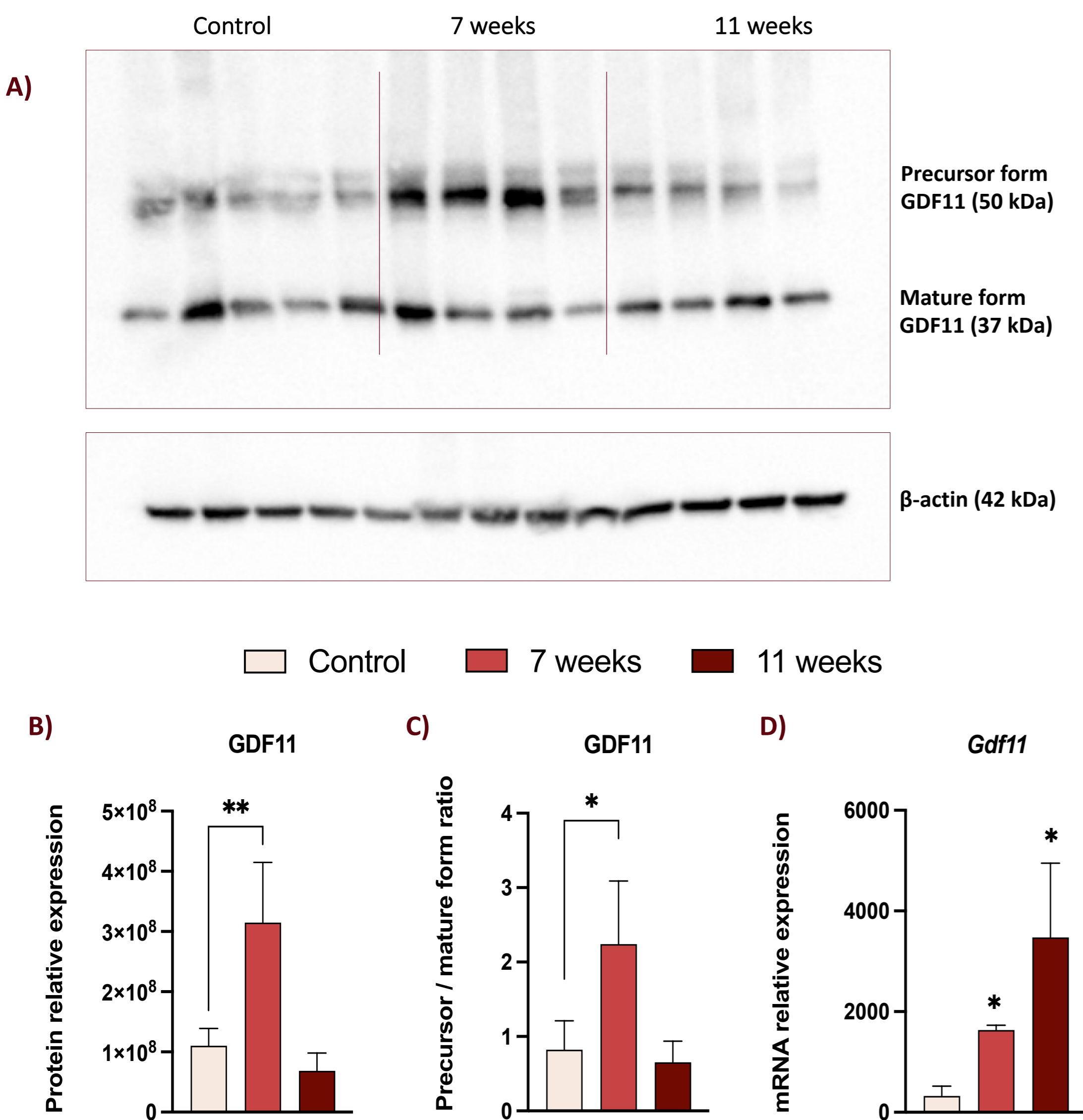
EXPERIMENTAL DESIGN
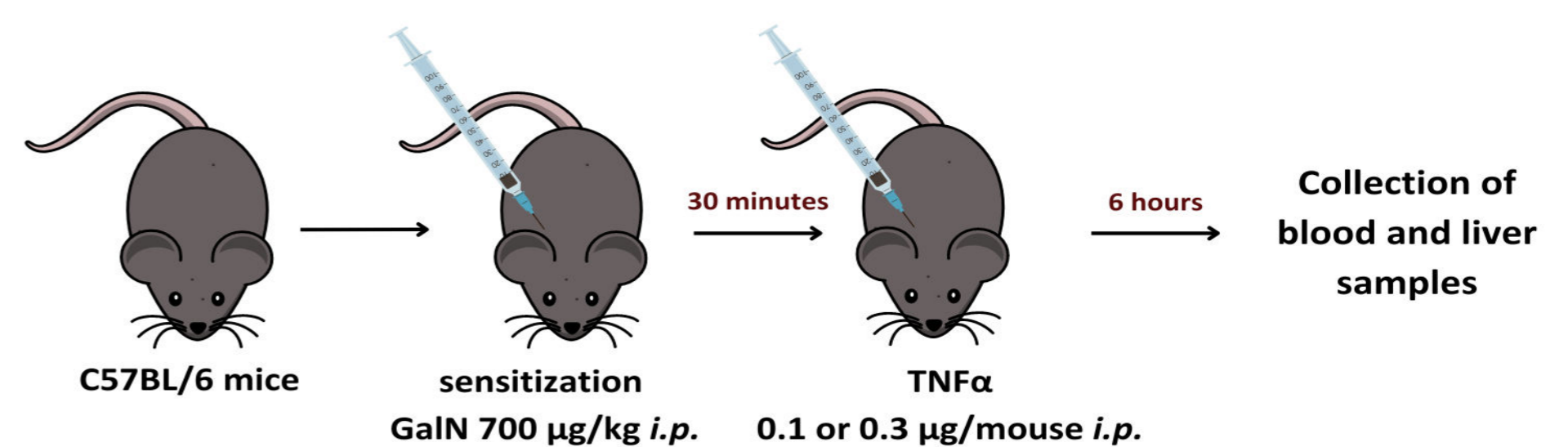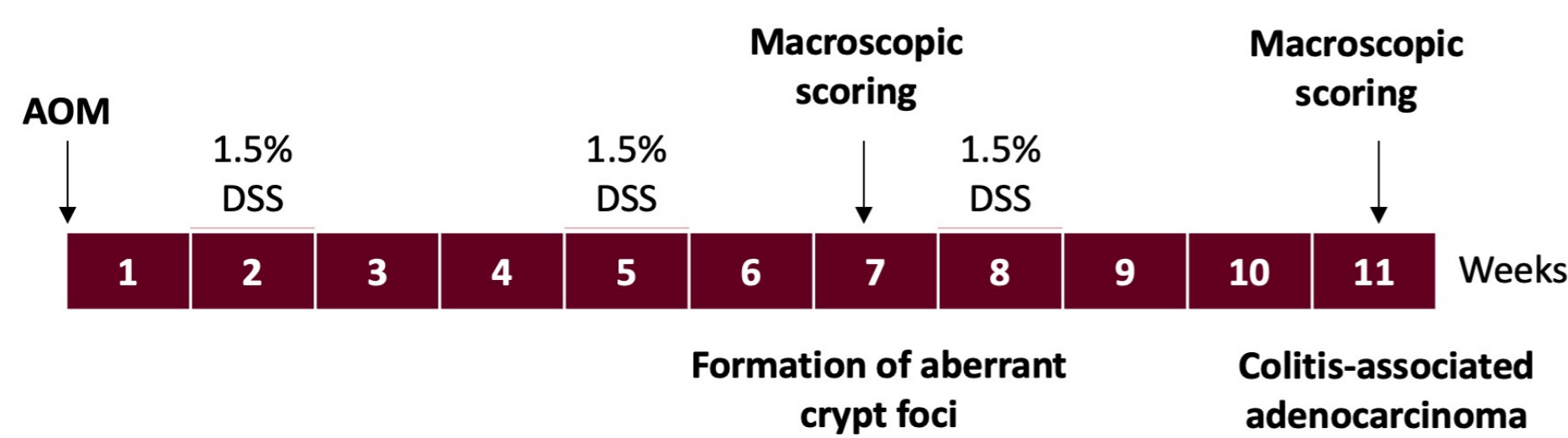
RESULTS

Fig. 1 Western blot of GDF11 precursor, cleaved form, and β-actin (A). Protein relative expression (B), relative precursor to mature form ratio (C), mRNA level (D) of GDF11 in the colon collected from mice with colitis-associated colorectal cancer. Statistical significance confirmed by t-Student test * $p < 0.05$, ** $p < 0.01$.
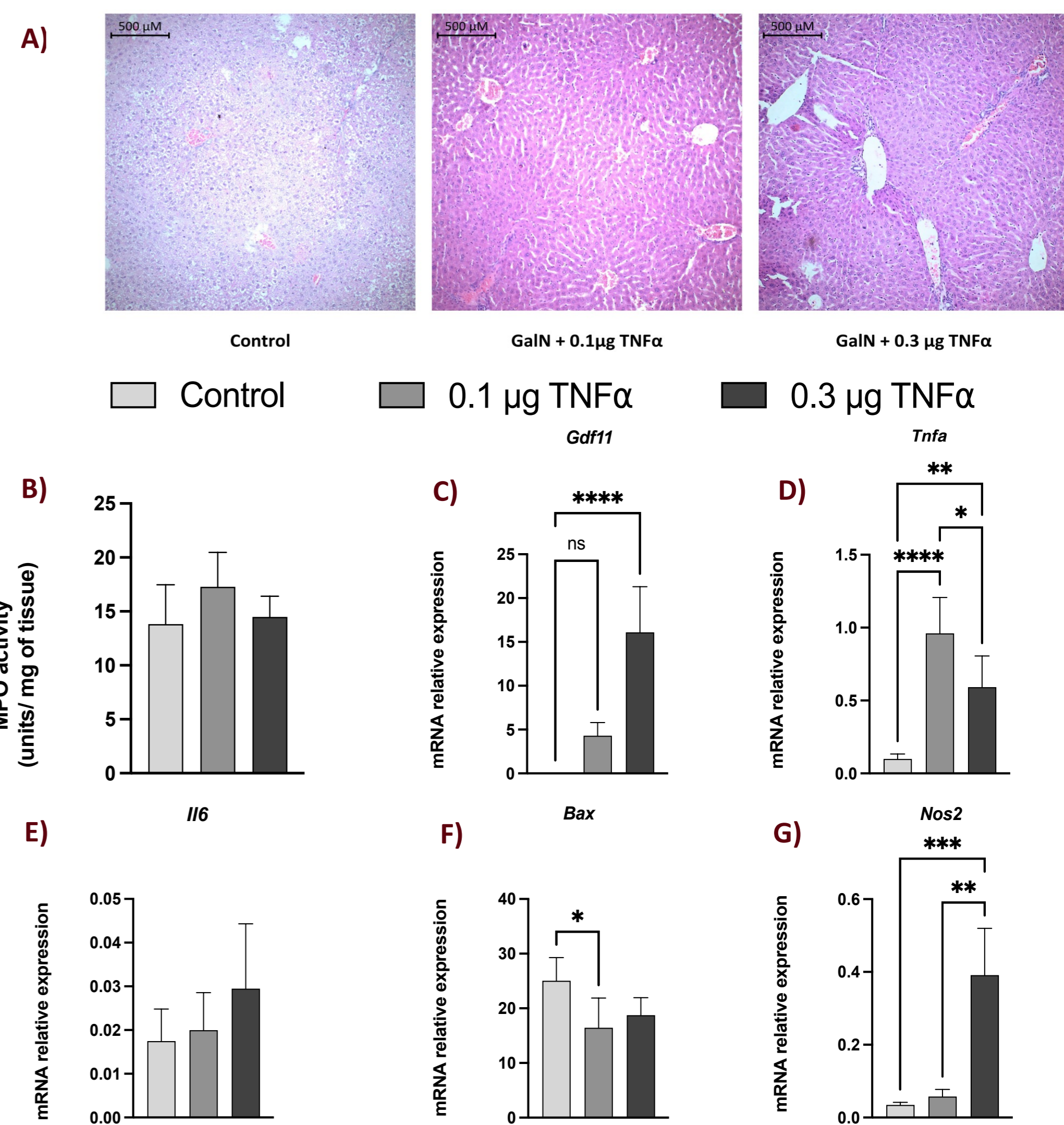

Fig. 2 Representative microscopy pictures of a healthy mouse liver and acute liver injury, original magnification ×100 (A). MPO activity measured in mouse liver (B), *Gdf11* (C), *Tnfα* (D), *Il-6* (E), *Bax* (F) *Nos2* (G) mRNA expression in the liver. Statistical significance confirmed with one-way ANOVA * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ ****$p < 0.0001$.

CONCLUSIONS

GDF11 expression differs according to the stage of the colitis-associated colorectal cancer in mice. The expression of the precursor form significantly increased at early stage of colitis-associated colorectal cancer development. We validated model of acute hepatitis in mice.