

# Novel Gubra Amylin NASH (GAN) diet-induced obese mouse models of biopsy-confirmed non-alcoholic steatohepatitis

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## INTRODUCTION AND AIM

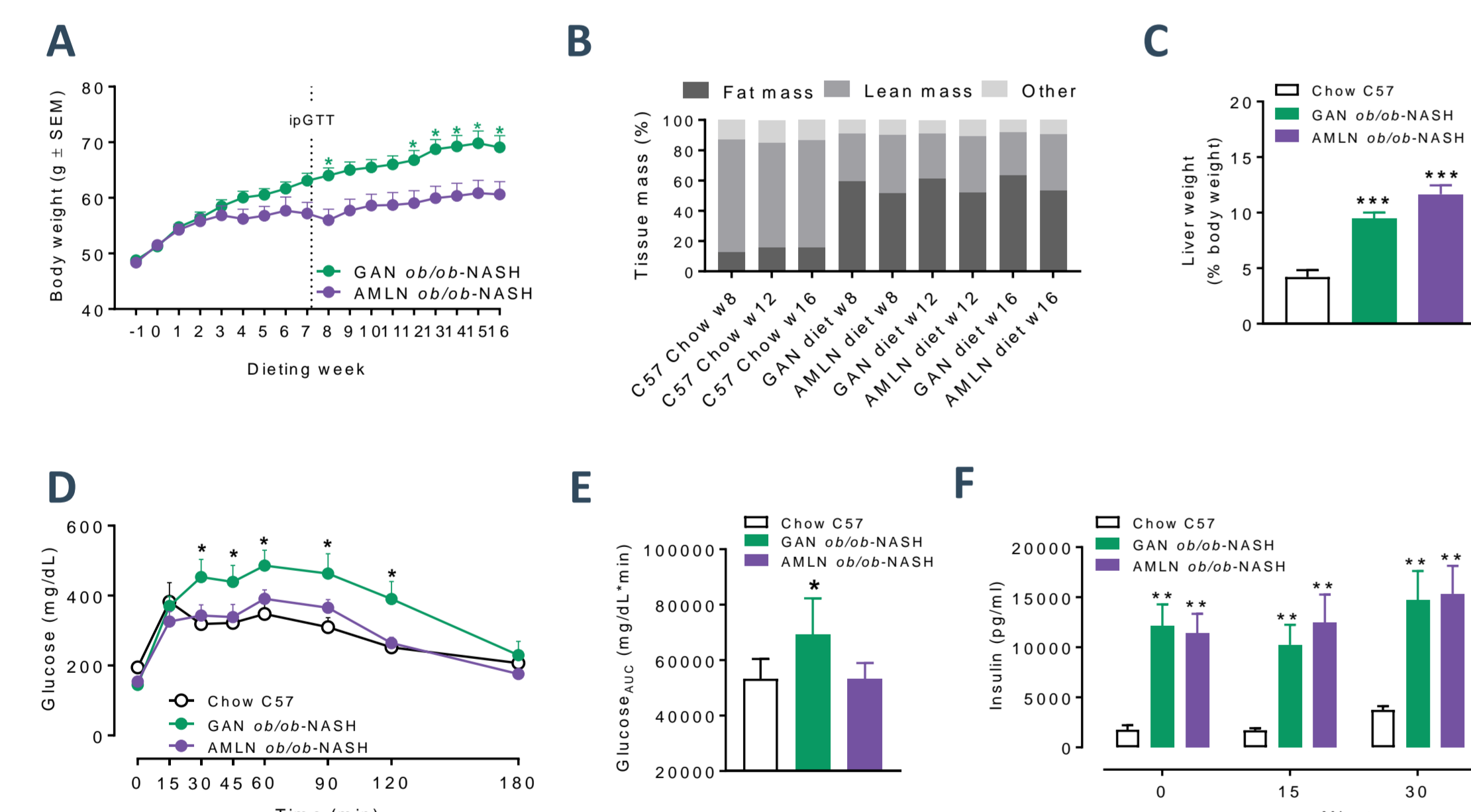
The Amylin Liver NASH (AMLN) diet-based *ob/ob* and C57BL/6J mouse models display clinical translatability with respect to key metabolic and liver biopsy-confirmed pathological changes associated with non-alcoholic steatohepatitis (NASH). A recent FDA ban on trans-fats in foods has prompted the development of a new NASH diet capable of promoting a compatible level of disease, as the AMLN diet contains trans-fat-containing Primex shortening. The present study aimed to assess the metabolic and liver pathological phenotype in *ob/ob* and C57BL/6J mice fed a palmitic acid-enriched high-fat diet with a nutrient composition and caloric content similar to the AMLN diet.

## METHODS

Male *ob/ob* mice were fed chow, AMLN diet (40% total fat kcal of which 18.5% were trans-fat kcal, 20% fructose, 2% cholesterol; Research Diets #D09100301) or a modified AMLN diet with Primex substituted by equivalent amounts of palm oil (Research Diets, #D09100310), termed Gubra Amylin NASH (GAN) diet, for up to 30 weeks. C57BL/6J mice were fed the same diets for 28 weeks. NAFLD activity score (NAS) and fibrosis staging was assessed. Quantitative histomorphometric analyses included fractional (%) area of steatosis (hematoxylin-eosin), inflammation (galectin-3), and collagen (Col1a1). RNA sequencing was performed on terminal liver samples.

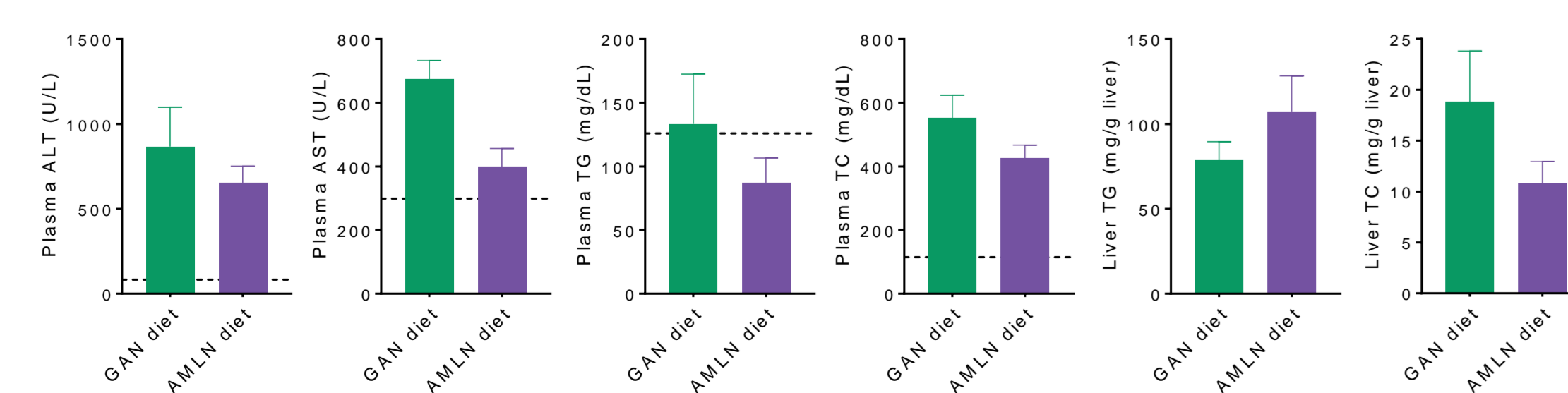
## RESULTS

### Weight gain, body composition and liver mass in *ob/ob*-NASH mice fed AMLN or GAN diet



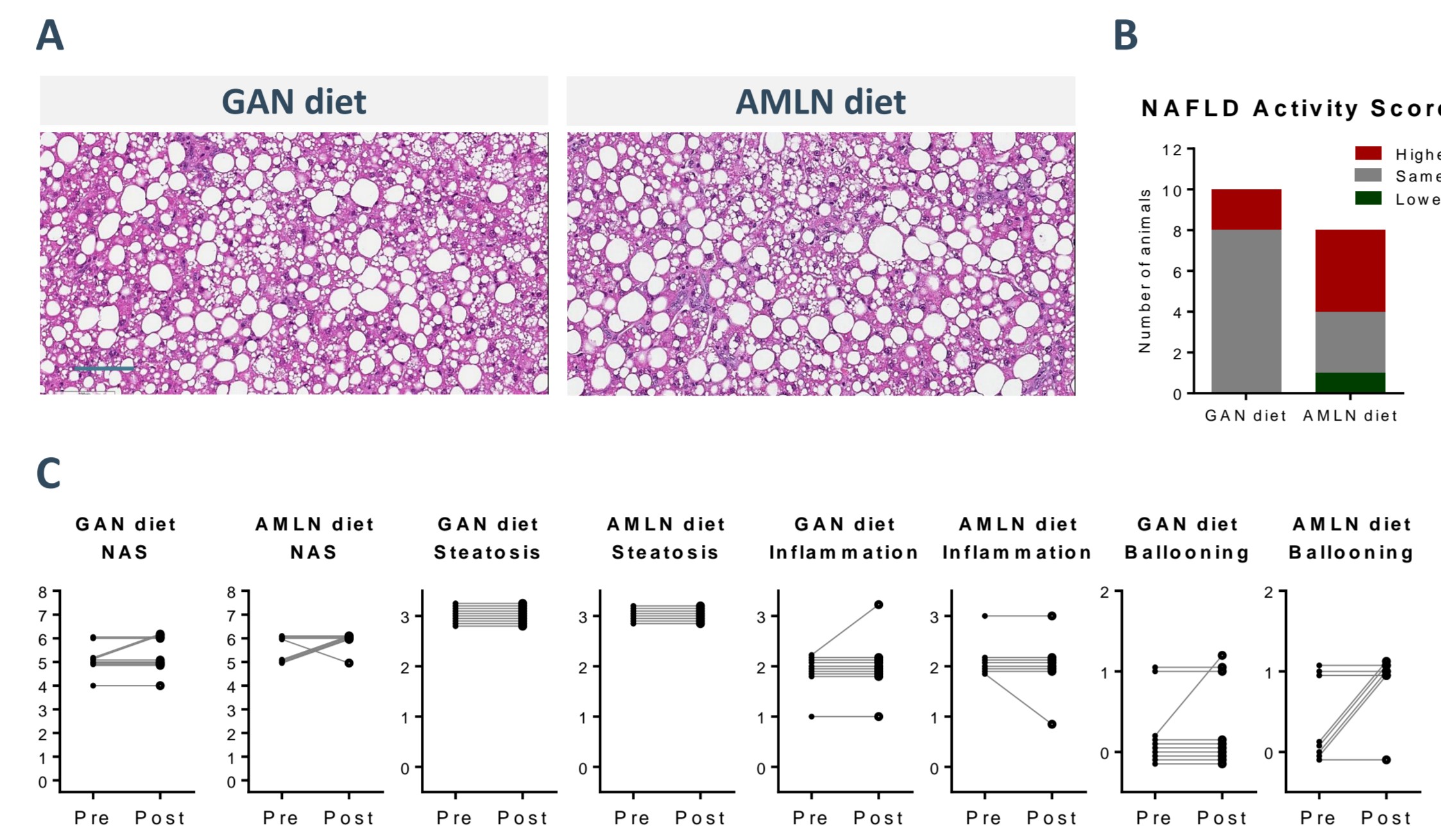
**Figure 1 |** Study 1 | Metabolic parameters in *ob/ob* mice fed AMLN (AMLN *ob/ob*-NASH) or GAN (GAN *ob/ob*-NASH) diet for 16 weeks. A) Body weight gain, B) body composition, C) terminal liver weight (week 16), D) ipGTT, E) AUC<sub>glucose</sub> (0-180 min), F) plasma insulin (0, 15, 30 min). ipGTT was performed in week 7 of the dieting period. \**p*<0.05, \*\**p*<0.01, \*\*\**p*<0.001 vs. chow-fed C57BL/6J (chow C57) control mice.

### Plasma and liver biomarker changes in *ob/ob*-NASH mice fed AMLN or GAN diet



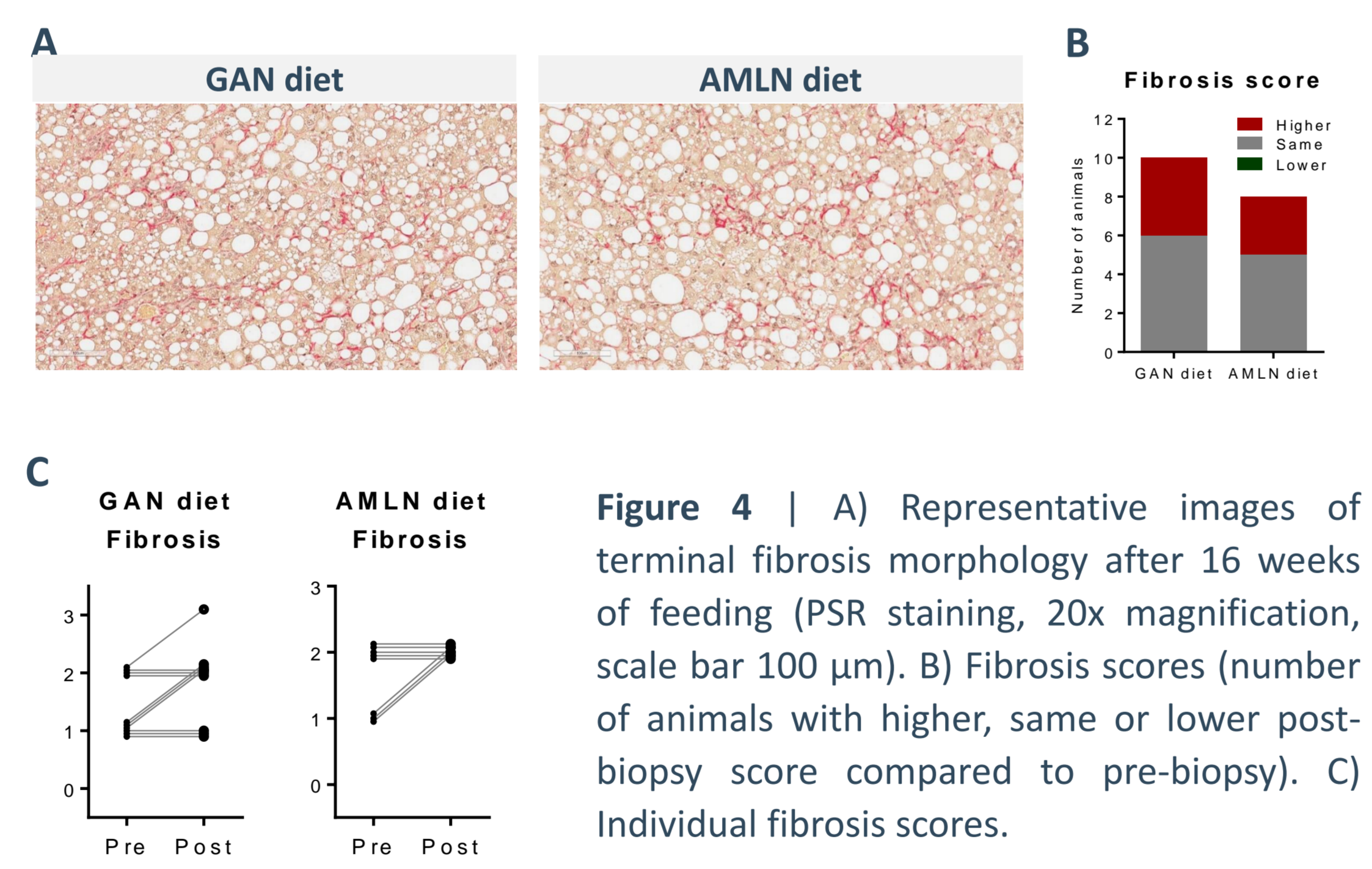
**Figure 2 |** Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), total triglycerides (TG), total cholesterol (TC) and liver lipids (TG, TC) in *ob/ob* mice fed AMLN or GAN diet for 16 weeks. Horizontal dotted line indicates corresponding level in age-matched chow-fed C57BL/6J mice.

### Comparable biopsy-confirmed NAFLD Activity Scores in *ob/ob*-NASH mice fed AMLN or GAN diet



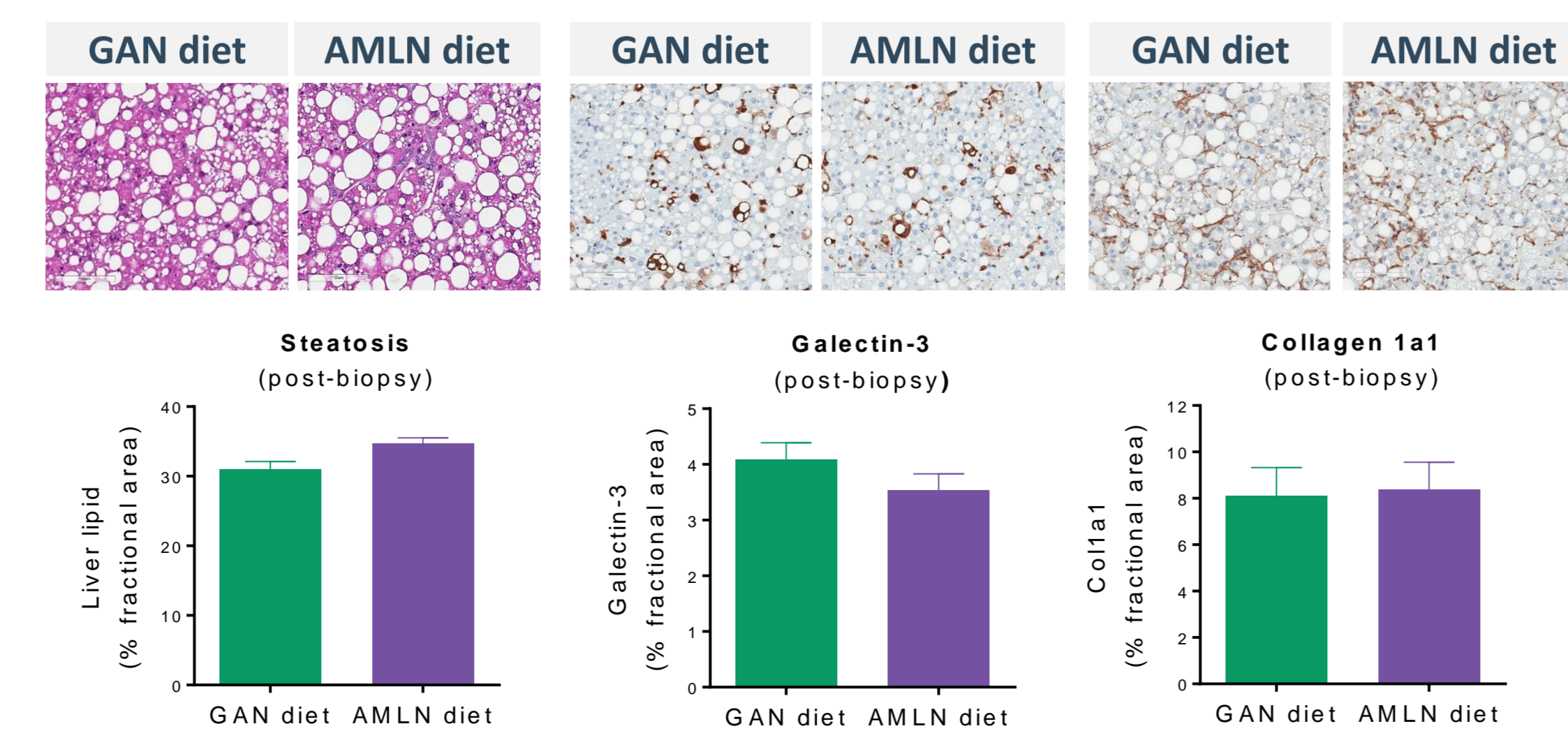
**Figure 3 |** A) Representative images of terminal liver morphology (HE staining, 20x magnification, scale bar 100  $\mu$ m). B) Composite NAFLD Activity Score (NAS, number of animals with higher, same or lower post-biopsy score compared to pre-biopsy). C) Individual NAS, steatosis, inflammation and ballooning scores. Paired pre- and post-biopsies were samples at 9 and 16 weeks of feeding, respectively.

### Comparable biopsy-confirmed fibrosis scores in *ob/ob*-NASH mice fed AMLN or GAN diet



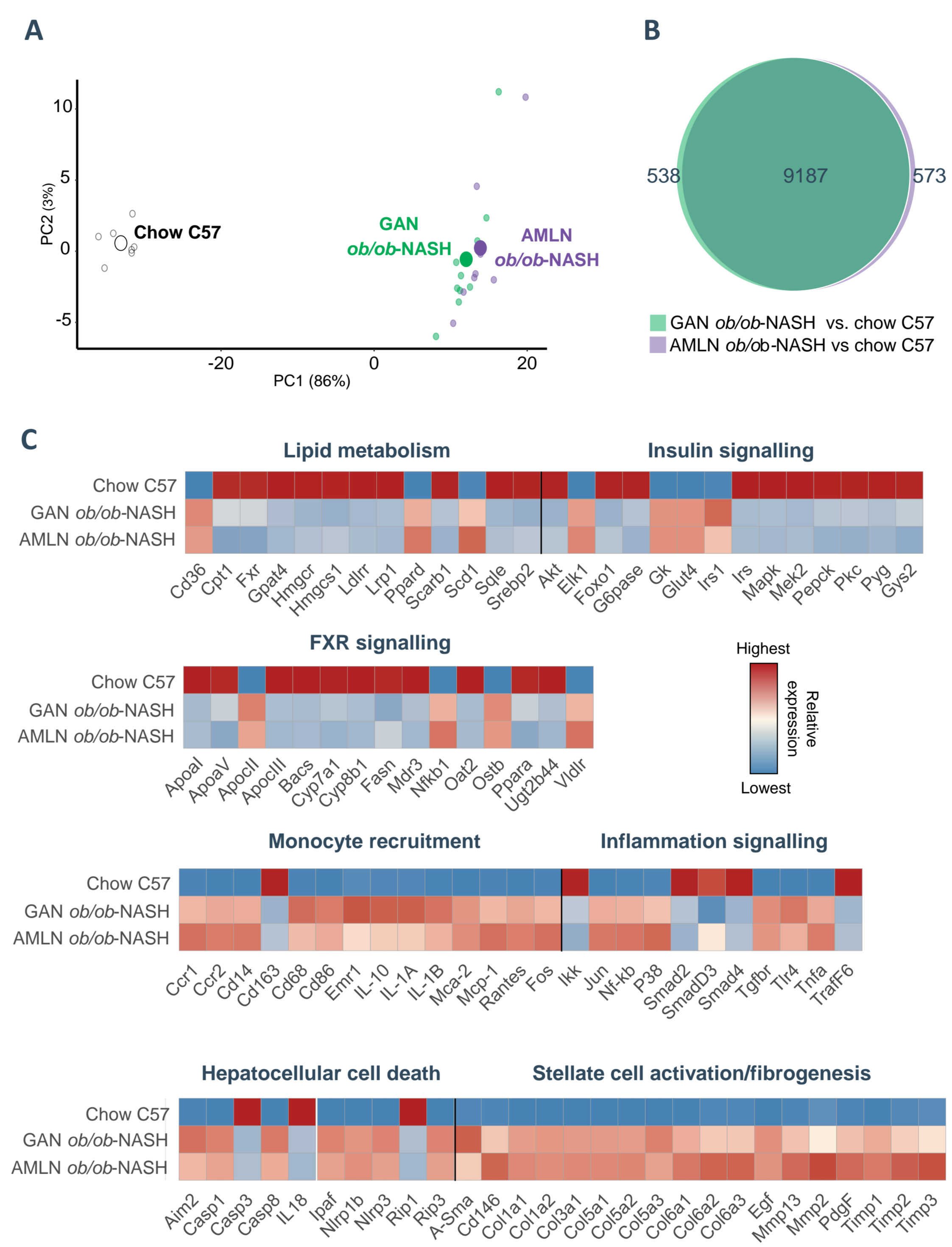
**Figure 4 |** A) Representative images of terminal fibrosis morphology after 16 weeks of feeding (PSR staining, 20x magnification, scale bar 100  $\mu$ m). B) Fibrosis scores (number of animals with higher, same or lower post-biopsy score compared to pre-biopsy). C) Individual fibrosis scores.

### Comparable quantitative histopathological changes in *ob/ob*-NASH mice fed AMLN or GAN diet



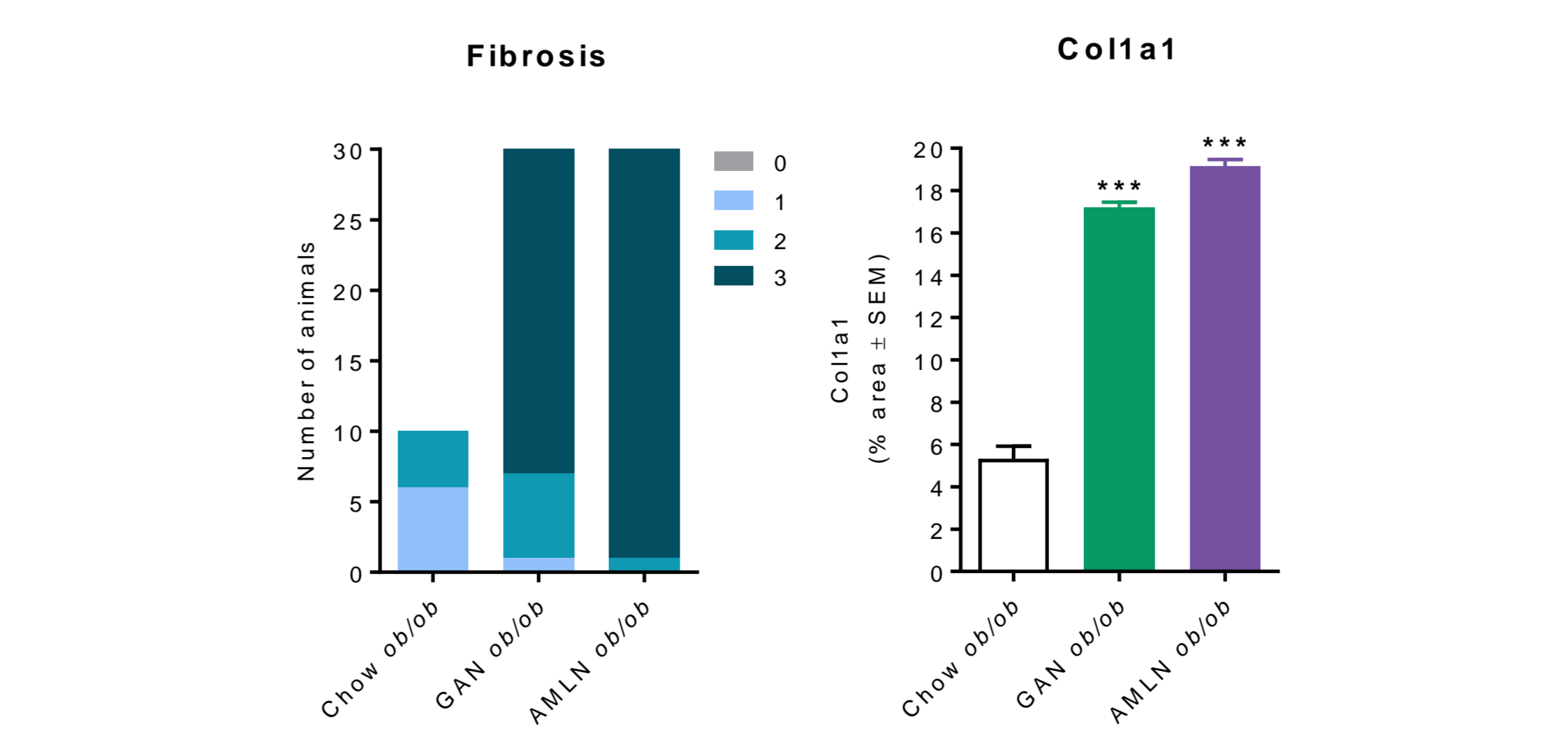
**Figure 5 |** Top panels: Representative images of terminal fibrosis morphology after 16 weeks of feeding. Lower panels: Fractional (%) area of steatosis (HE staining), inflammation (Galectin-3 IHC) and fibrosis (Col1a1 IHC) determined by imaging-based histomorphometry. Scale bar 100  $\mu$ m.

### Comparable liver transcriptome changes in *ob/ob*-NASH mice fed AMLN or GAN diet



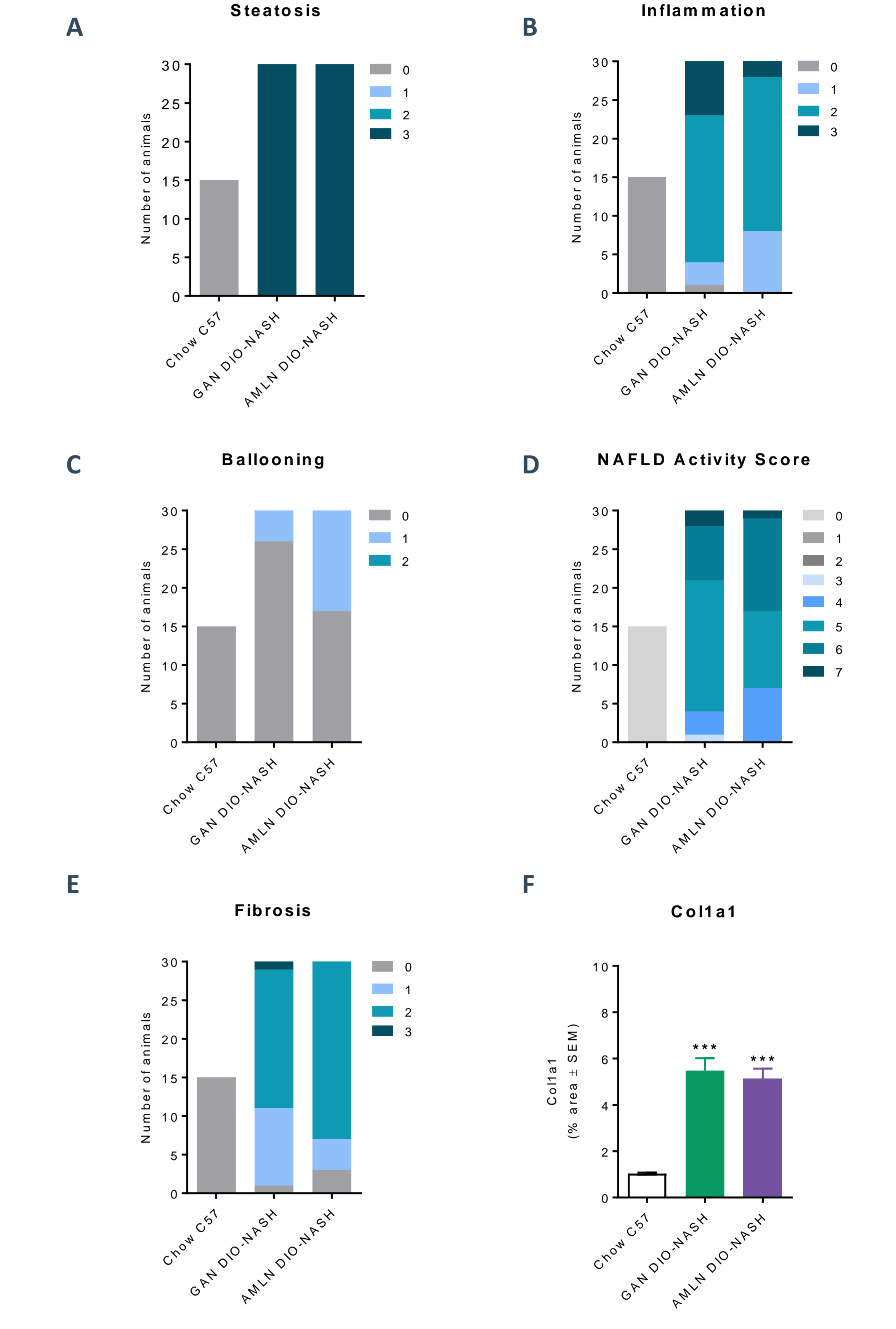
**Figure 6 |** RNA sequencing. Hepatic gene expression profiles in chow-fed C57BL/6J mice (Chow C57), AMLN and GAN *ob/ob*-NASH mice after 16 weeks of feeding. A) Principal component analysis (PCA) of samples based on top 500 most variable gene expression levels. B) Group-wise comparison of the total number of differentially expressed genes between GAN and AMLN *ob/ob*-NASH mice vs. chow-fed C57BL/6J mice. C) Relative gene expression levels (z-scores) of differentially regulated candidate genes associated with NASH and fibrosis.

### High rates of bridging fibrosis in *ob/ob*-NASH mice fed AMLN or GAN diet for extended periods



**Figure 7** Terminal liver histopathology in *ob/ob* mice fed chow (n=10 mice), AMLN (AMLN DIO-NASH, n=30 mice) or GAN (GAN DIO-NASH, n=30 mice) diet for 30 weeks. Left panel, histopathological scoring of fibrosis. Right panel, fractional (%) area of collagen-1a1. \*\*\**p*<0.001 vs. chow-fed *ob/ob* mice.

### Comparable liver histopathology in DIO-NASH mice fed AMLN or GAN diet



**Figure 8 |** Terminal liver histopathology in C57BL/6J mice fed chow (n=15 mice), AMLN (AMLN DIO-NASH, n=30 mice) or GAN (GAN DIO-NASH, n=30 mice) diet for 28 weeks. Histopathological scores of steatosis (A), lobular inflammation (B), hepatocyte ballooning (C), composite NAFLD Activity Score (NAS, D), and fibrosis (E). Fractional (%) area of collagen-1a1 (F). \*\*\**p*<0.001 vs. chow-fed C57BL/6J mice.

## CONCLUSIONS

- Modification of the AMLN diet by substitution of Primex shortening with palm oil (GAN diet) results in a maintained NASH phenotype in both *ob/ob*-NASH and DIO-NASH mice.
- Compared to the AMLN diet, the GAN diet promotes further body weight gain and impairs glucose intolerance in *ob/ob*-NASH mice.
- The clear metabolic and histopathological hallmarks of fibrotic NASH in *ob/ob*-NASH and DIO-NASH mice fed the GAN diet highlight the suitability of this model for characterizing novel drug therapies for NASH.