Automated AI-assisted assessment of NAS and fibrosis stage in biopsy-confirmed rodent models of MASH

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Background & Aim

NAFLD Activity Scoring (NAS) and fibrosis staging is widely used in clinical trials and preclinical studies for metabolic dysfunction-associated steatohepatitis (MASH). The present study aimed to develop and validate an automated deep learning-assisted digital imaging analysis pipeline, termed **GHOST (Gubra Histopathological Objective Scoring Technology)** for objective assessment of NAS and fibrosis stage in rodent models of MASH.
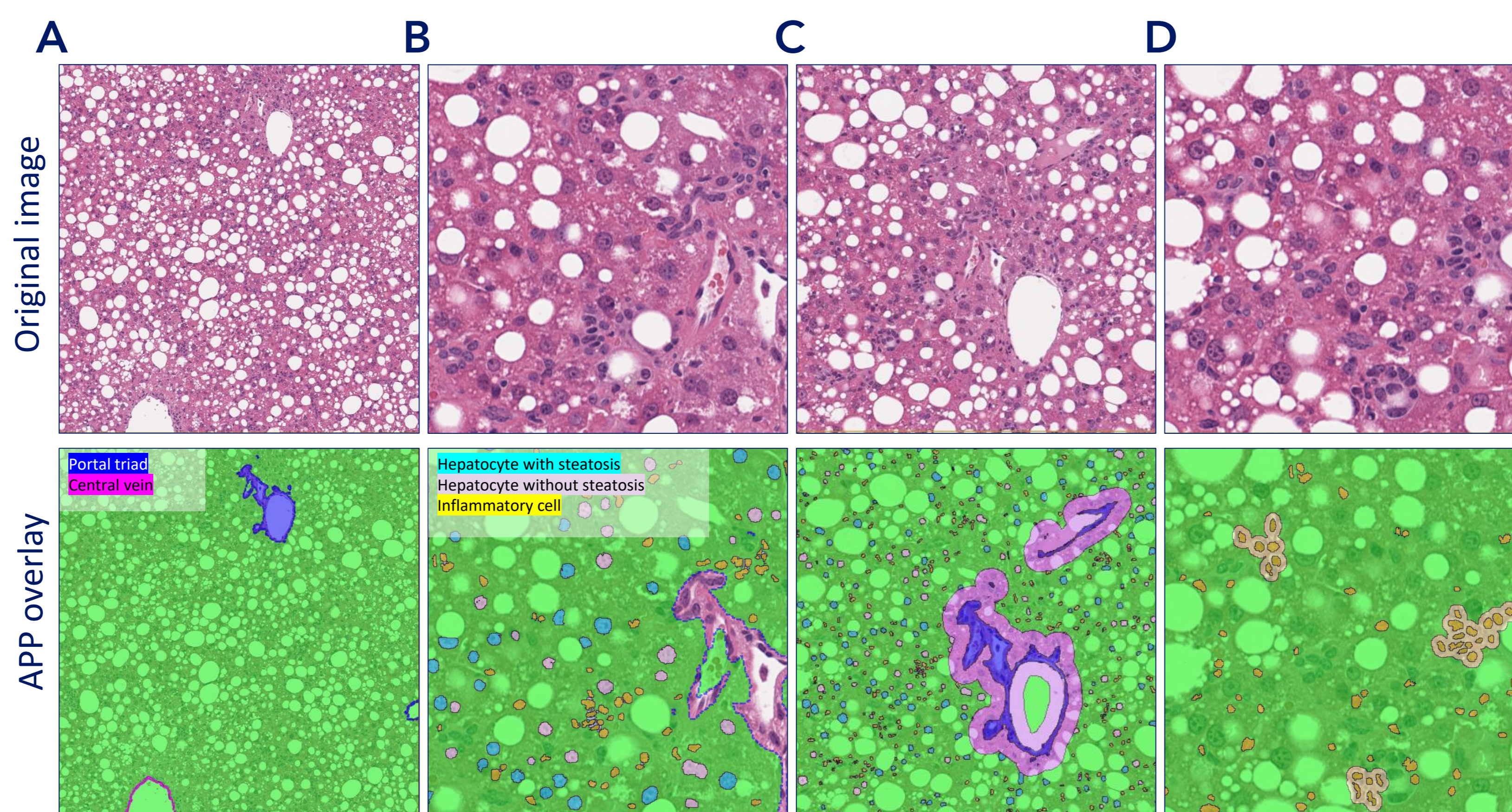
Methods

Liver biopsies were obtained from GAN DIO-MASH mice (after being fed GAN diet for 28-35 weeks) and CDAA-HFD rats (fed the CDAA-HFD diet for 12 weeks). Chow-fed control mice and rats served as controls. GHOST was applied to HE and PSR stained sections for the assessment of NAS (n=338 mice) and fibrosis stage (Kleiner classification, n=537 mice). GHOST was extended to perform fibrosis scoring (Ishak classification) on PSR-stained sections from CDAA-HFD rats (n=86). All GHOST data were validated against manual scoring performed by expert histopathologists.

Conclusion

+ GHOST shows high agreement with manual scoring by expert histopathologist in industry-standard rodent models of MASH.

+ GHOST provides unbiased, fast, accurate and reproducible histopathological scoring.

+ GHOST enables quantitative analysis of scoring-derived variables.

+ GHOST is highly applicable for assessment of drug effects on clinical histopathological hallmarks in mouse and rat models of MASH.
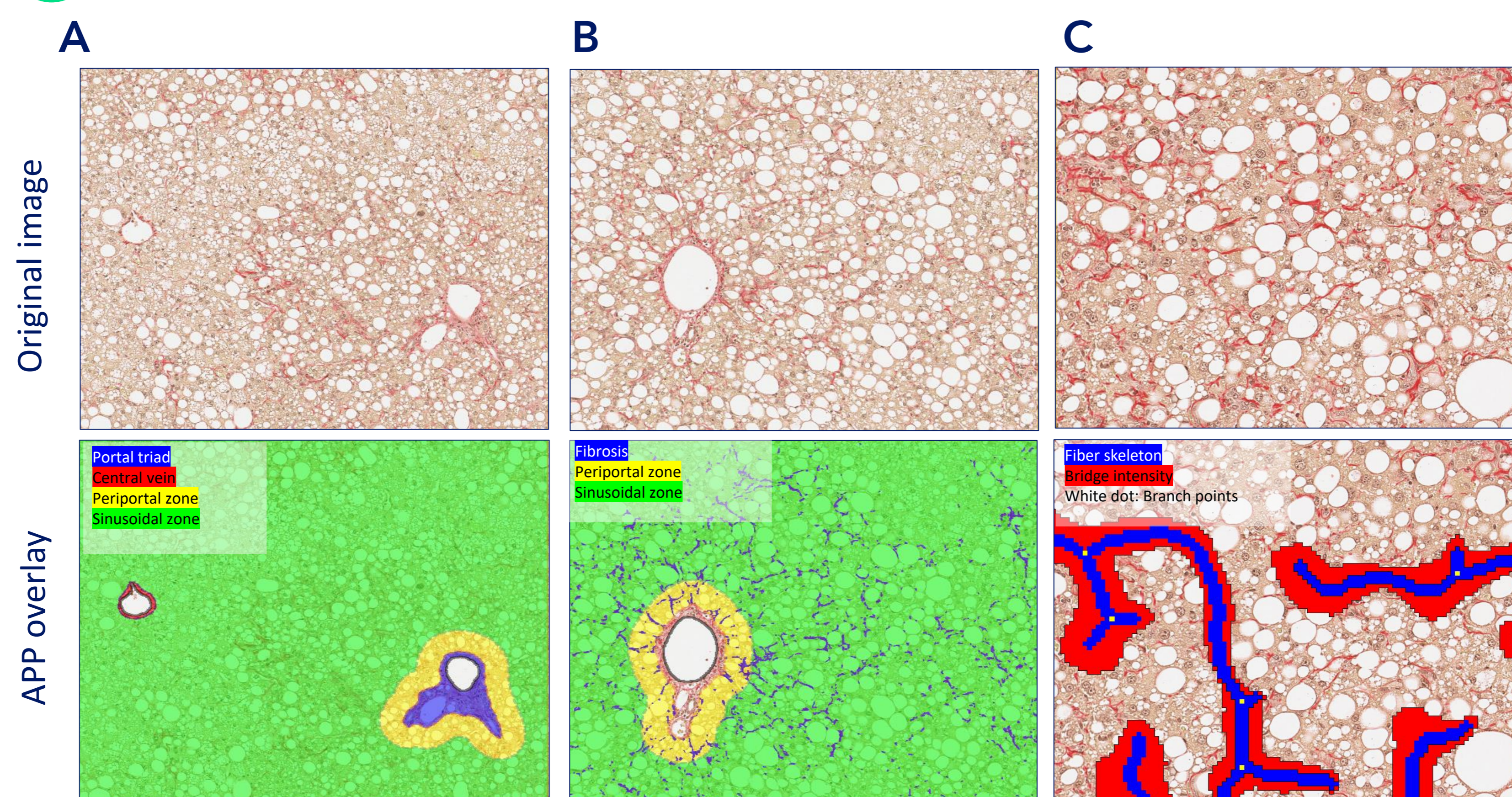
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1 NAFLD Activity Score using GHOST

Figure 1. GHOST-based NAFLD Activity Score (NAS). (A) Portal triads and central veins were detected using deep learning (10X). (B) Deep learning detected nuclei of hepatocytes with steatosis, hepatocytes without steatosis, and inflammatory cells (20X). (C) Post-processing excludes periportal inflammation. (D) Post-processing converted clusters of ≥4 inflammatory cells into foci. Scores were calculated based on simple threshold. (E) Correlation of GHOST and manual scoring. Cohen's Kappa value = 0.72
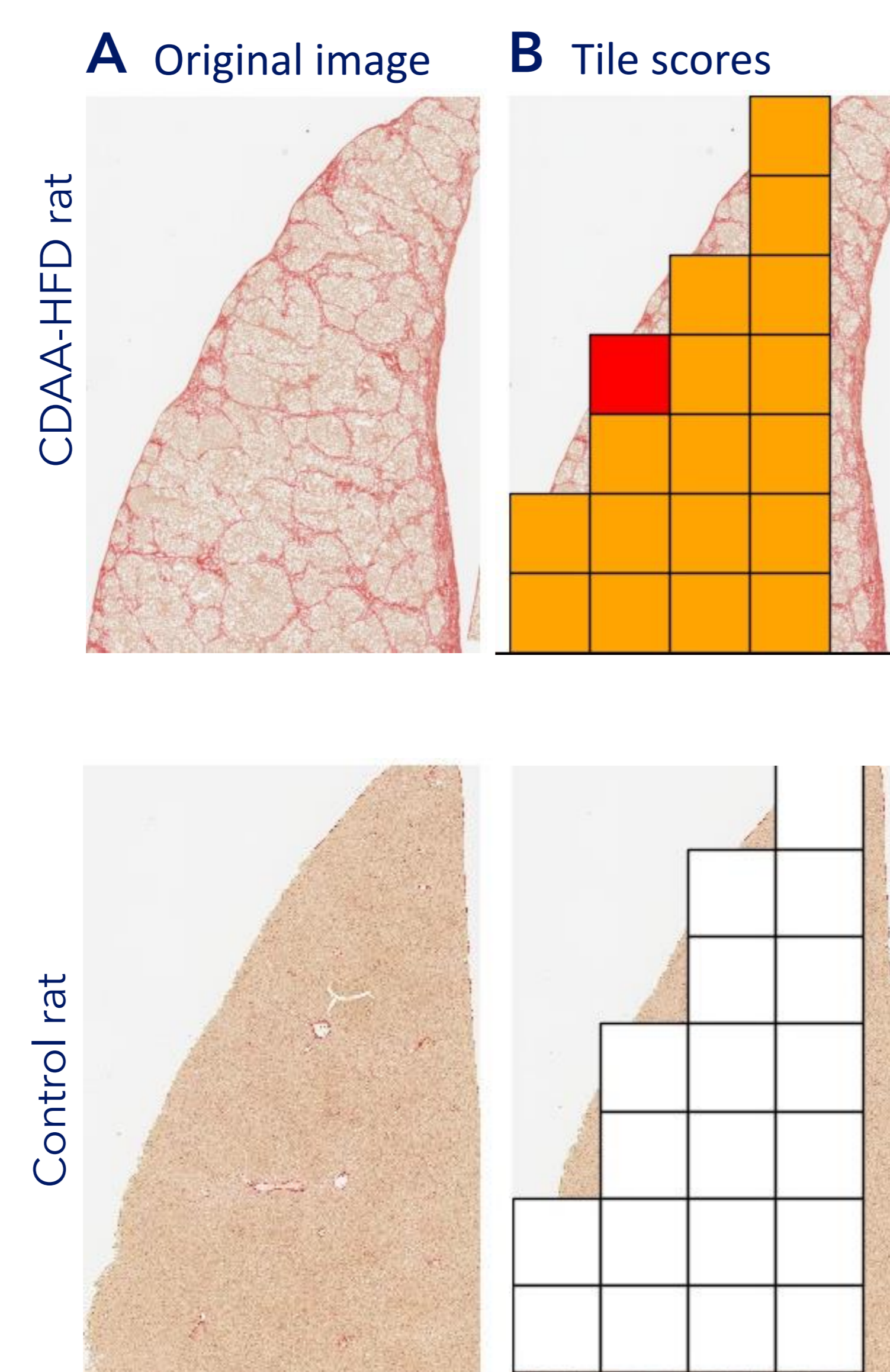
NAFLD Activity Score

Manual score vs GHOST score

	0	1	2	3	4	5	6	7
0	12	9	2	0	0	0	0	0
1	1	3	4	0	0	0	0	0
2	0	2	10	0	0	0	0	0
3	0	0	2	12	4	0	0	0
4	0	0	3	11	12	3	0	0
5	0	0	0	9	15	80	45	0
6	0	0	0	0	4	21	64	0
7	0	0	0	0	0	0	0	1

2 Fibrosis Staging using GHOST

Figure 2. GHOST-based fibrosis scoring. (A) Portal triads and central veins were detected using deep learning (post-processing creates a periportal zone of 100 μm). (B) Fibrosis was detected using the linear Bayesian image analysis method in the periportal and sinusoidal zones, and different measures of collagen fiber fragment size and shape was used to predict bridging. (C) Bridging was also detected using the Threshold image analysis method based on a polynomial local linear filter feature. (D) Correlation of GHOST and manual scoring. Cohen's Kappa value = 0.84

Fibrosis Stage

Manual score vs GHOST score

	0	1	2	3
0	61	7	0	0
1	6	53	23	0
2	0	16	176	22
3	0	0	29	144

3 Ishak Fibrosis Score using GHOST

Figure 3. GHOST-based Ishak fibrosis score. (A) Original image of PSR-stained liver section from CDAA-HFD rat (top) and age-matched control (bottom). (B) Images are divided into squares and classified using convolutional neural network (CNN) analysis. Output of the CNN analysis was used in a machine learning algorithm to train GHOST to predict fibrosis stage. Boxes of different colours have been given different Ishak scores : White=0, orange=5 and red=6. (C) Correlation of GHOST and manual scoring. Cohen´s Kappa value=0.82

Ishak Fibrosis Score

Manual score vs GHOST score

	0	1	2	3	4	5	6
0	11	1	0	0	0	0	0
1	2	1	0	0	0	0	0
2	0	0	2	3	1	0	0
3	1	0	6	8	1	0	0
4	0	0	0	1	9	2	0
5	0	0	0	0	4	8	10
6	0	0	0	0	1	3	20

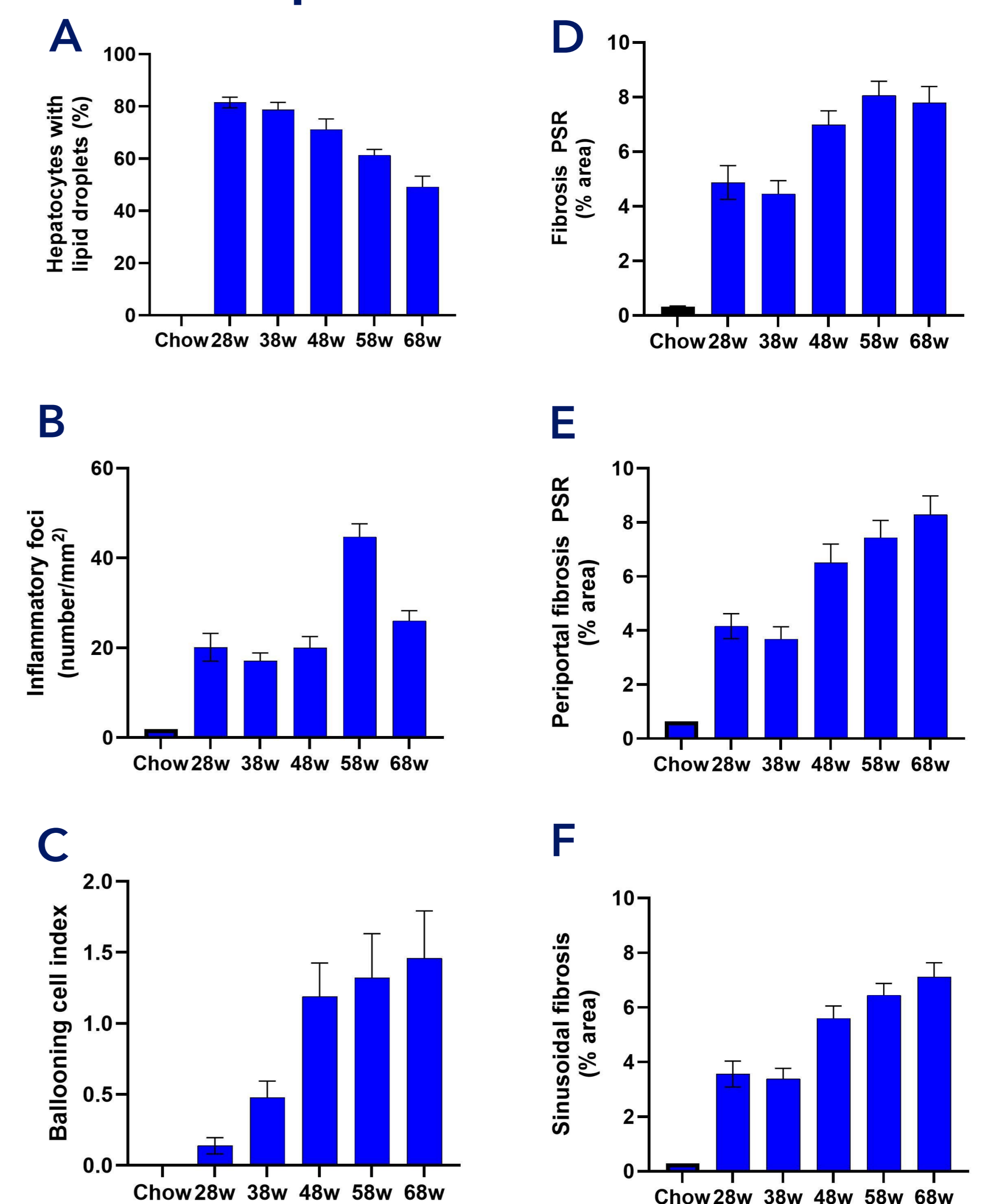## 4 Histomorphometric variables

Figure 5. GHOST-based histomorphometrics on scoring variables. (A) Percentage of hepatocytes with lipid droplets relative to total hepatocyte counts (mean ±SEM) (B) Number of inflammatory foci pr mm² (mean ±SEM). (C) Ballooning cell index. (D) Percentage of area with fibrosis in section (mean ±SEM). (E) Percentage of area of periportal fibrosis in the section (mean ±SEM). (F) Percentage of area of sinusoidal fibrosis in the section (mean ±SEM).