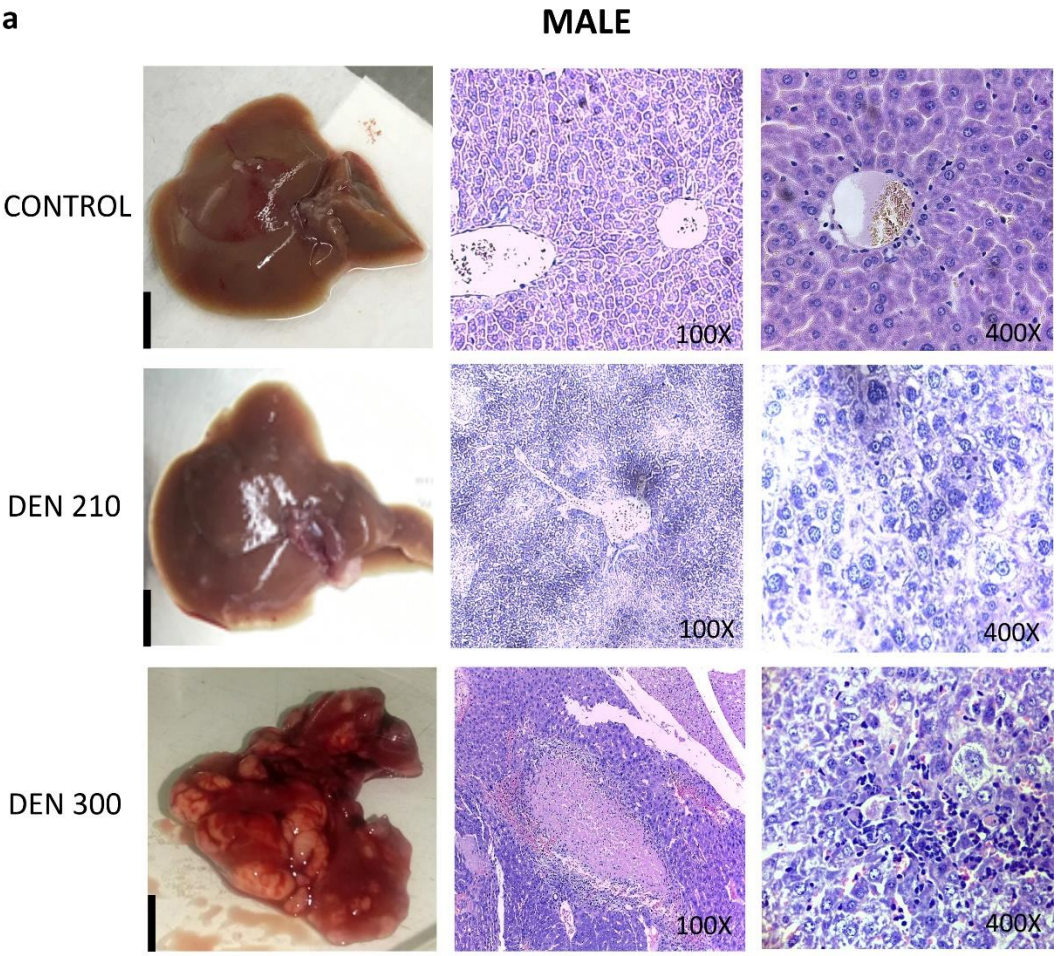
SUPPLEMENTARY FIGURES



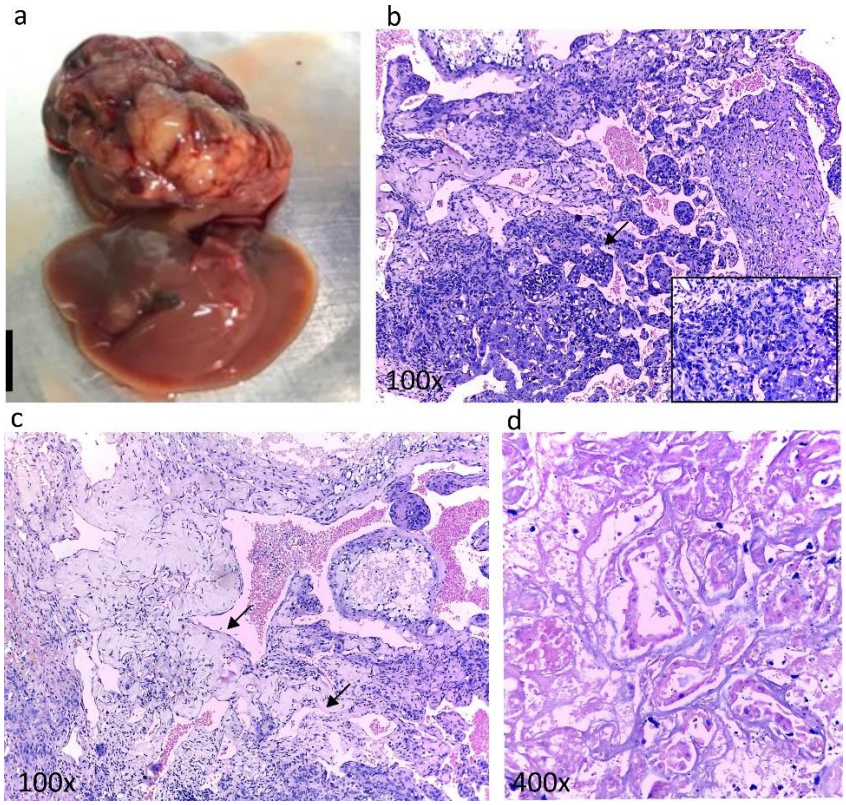
d

	C	D210	D300
Average N° Mitosis for field	0-1	1-2	2-3
Average Kupfer cells for field	5	18	60

Supplementary 1: Development of liver tumors induced by diethylnitrosamine (DEN) in male C3H mice 210- and 300-days post-DEN.

(a) Representative macroscopic and histopathological images of male C3H control livers 210- and 300-days post-DEN inoculation. Haematoxylin and eosin (H&E) stainings, 100 and 400X.

Day 210 post DEN: the image shows hepatic adenoma. The nuclei appear small and contain clumped chromatin. The cytoplasm displays vacuoles and primarily exhibits basophilic material (400x). Day 300 post DEN: Note a nodular proliferation of hepatocytes, appearing trabecular. Within the proliferation, focal areas of coagulative necrosis are observed. The neoplastic cells compress the adjacent parenchyma. The cytoplasm is basophilic, with some areas exhibiting vacuolation. **(b)** Heat map represents the percentages of hyperplasia, adenomas, and carcinomas diagnosed within each experimental group of males, including controls, and at days 210 and 300 post-DEN treatment. **(c)** Detailed information on the degree of cellular atypia, distortion of liver architecture, and necrosis in DEN-treated males. The heat map indicates a semiquantitative analysis of the respective liver histologic features, ranging from faint red (not present) to dark red (a feature present in all/almost all liver sections). **(d)** Average numerical values for mitosis and Kupffer cells per field. Ten fields per liver were analyzed. Due to the lack of significant histological changes observed in the control groups euthanized on day 210 and day 300, the data from the controls on day 210 is depicted in the figure for simplification purposes.



Supplementary 2: Macroscopic image of HCC in DEN-treated female mouse at 300 days post-DEN inoculation.

(a) Macroscopic image of female DEN-treated liver. Note the large associated tumor mass (black bars: 5 mm). **(b)** Cell proliferation with high pleomorphism and cytoplasmic vacuolation (arrow, box). **(c)** Multiple areas of vascular proliferation are seen (arrows). **(d)** Areas of necrosis between hepatocytes. Haematoxylin and eosin (H&E) staining, 100 and 400X.

a

Characteristic (%)	Female		Male	
	C210	C300	C210	C300
Cellular Atypia	4.5 (±2.5)	4.0 (±3.1)	5.0 (±2.5)	5.0 (±2.1)
Distorted Architecture	0.4 (±0.2)	0.4 (±0.5)	0.3 (±0.2)	0.4 (±0.5)
Necrosis	0.0 (±0.5)	0.0 (±0.5)	0.0 (±0.2)	0.0 (±0.1)

b

Characteristic (%)	*Female*		
	C	D210	D300
Cellular Atypia	4.0 (±3.1)	14.0 (±5.5) ns.	71.0 (±8.5) ****
Distorted Architecture	0.4 (±0.5)	5.8 (±3.1) ns.	55.0 (±10.0)****
Necrosis	0.0 (±0.5)	5.8 (±3.1) ns.	47.0 (±10.9)****
		# C vs. D210	*C vs. D300

c

Characteristic (%)	*Male*		
	C	D210	D300
Cellular Atypia	5.0 (±2.1)	47.5 (±15.0) ####	96.0 (±4.1) ****
Distorted Architecture	0.4 (±0.5)	56.0 (±15.5) ####	69.0 (±9.7) ****
Necrosis	0.0 (±0.1)	11.0 (±4.1) ns.	63.0 (±9.7) ****
		# C vs. D210	*C vs. D300

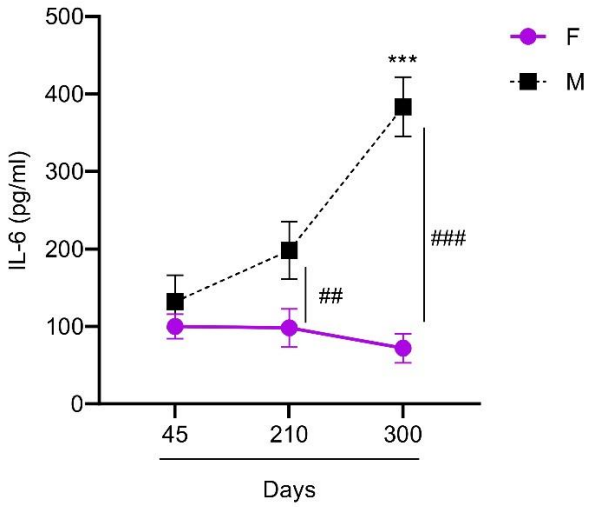
d

Characteristic (%)	D210		D300	
	Female	*Male*	*Female*	*Male*
Cellular Atypia	**** (p<0.0001)		**** (p<0.0001)	
Distorted Architecture	**** (p<0.0001)		* (p<0.01)	
Necrosis	ns (p>0.05)		** (p<0.001)	

Supplementary 3: Detailed information on the average numerical values of the respective liver histologic sections analyzed.

Values of the respective liver histologic features are detailed. The characteristic analysis includes cellular atypia distortion of liver architecture, and necrosis in control or DEN-treated female and male mice. **(a)** Detailed information of control female and male mice at days 210 and 300. Statistical comparison between control groups: ns. p >0.05. **(b)** Detailed information on female mice at days 210 and 300. Statistical comparison between control and experimental groups: ns. p >0.05; **** p < 0.0001. **(c)** Detailed information on male mice at days 210 and 300. Statistical

comparison between control and experimental groups: ns. p >0.05; #### p < 0.0001; **** p < 0.0001. The results were expressed as the percentage of occurrence of each characteristic per analyzed section of each evaluated representative hepatic lesion. Data represent the mean ± SEM of the percentage of the characteristic (%) of 5-7 mice per experimental group. **(d)** Statistical comparisons between females and males at days 210 and 300 for the different characteristics evaluated. ns. p >0.05; * p < 0.01 ** p < 0.001; **** p < 0.0001.



Supplementary 4: IL-6 levels of liver lysates in female and male mice at different times of life.

The concentration of IL-6 (pg/ml) in liver lysates in control female and male mice at 45, 210 and 300 days of life was evaluated by ELISA assay. Statistical comparison between experimental groups and control*: *** p < 0.001*. Statistical comparison among female and male groups at the same time: *## p< 0.01; ###: p < 0.001.*