Androgen aggravates liver fibrosis by activation of NLRP3 inflammasome in CCl_4 induced liver injury mouse model

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Supporting Material

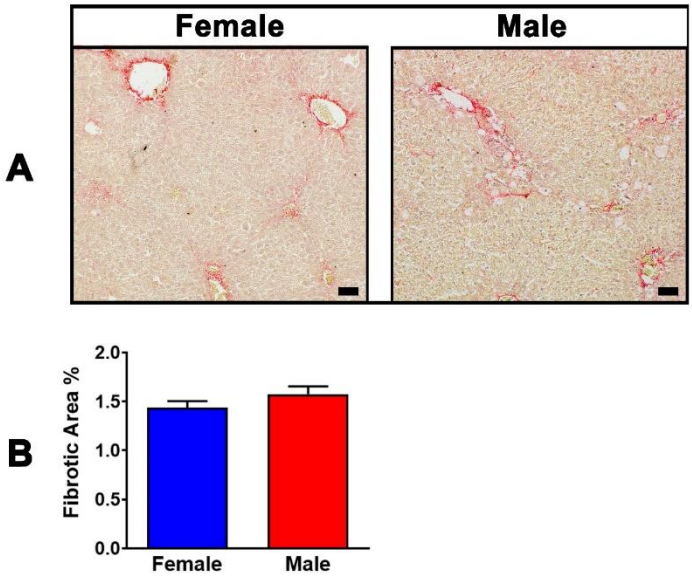

Figure S1. Assessment of liver fibrosis by Sirius staining of in CCl_4 treated mice

All mice received 0.2% CCl_4 treatment at the dosage of 10 mL/kg body weight once every two days for 2 weeks. (A) Sirius Red staining of female and male mice liver sections. (B) Quantification of Sirius red staining area in liver sections from female and male mice (t test, p= 0.114). (n = 5 individuals per group. For all statistical results, the data are shown as the means ± SD. Scale bars = 50 μm.).

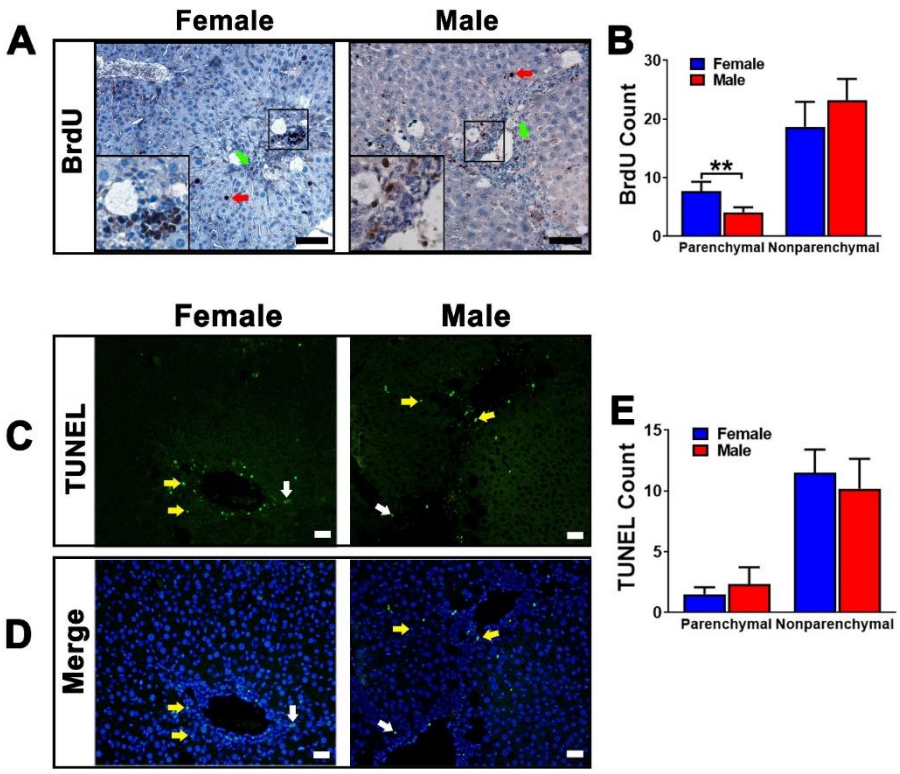

Figure S2 Cell proliferation and apoptosis in the liver of CCl_4 treated mice.

All mice received 0.2% CCl_4 treatment at the dosage of 10mL/kg body weight once every two days for 2 weeks. (A) 5-BrdU immunostaining of female and male mice liver sections. The red arrows highlight 5-BrdU positive parenchymal cells, and the green arrows highlight 5-BrdU positive nonparenchymal cells. (B) Quantitative analysis of 5-BrdU positive cells in the liver sections of female and male mice (t test, Parenchymal p=0.002, Nonparenchymal p=0.071). (C, D) TUNEL analysis of female and male mice liver sections (C) and merged with DAPI stain (D). The white arrows highlight TUNEL positive parenchymal cells, and the yellow arrows highlight TUNEL positive nonparenchymal cells. (E) Quantitative analysis of TUNEL positive cells in liver sections of female and male mice (t test, Parenchymal p=0.188, Nonparenchymal p=0.252). (n=5 individuals per group. For all statistical results, the data are shown as the means ± SD. **p< 0.01. Scale bars=50 μm).

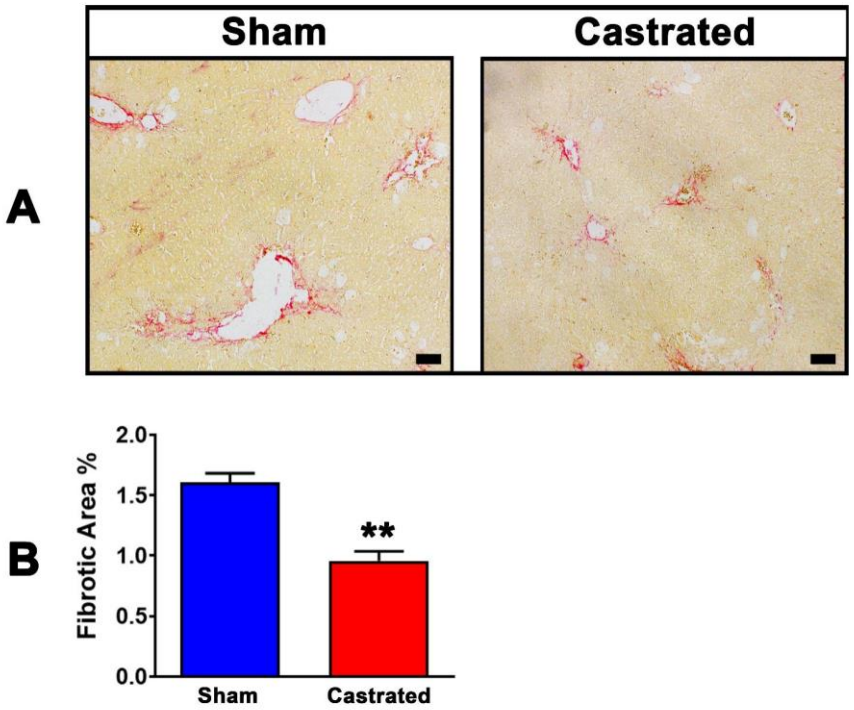

Figure S3. Assessment of liver fibrosis by Sirius staining of in CCl_4 treated mice

All mice received 0.2% CCl_4 treatment at the dosage of 10mL/kg body weight once every two days for 2 weeks. (A) Sirius Red staining of sham-operation and castrated male mice liver sections. (B) Quantification of Sirius red area in liver sections from sham-operation and castrated male mice (t test, $p< 0.001$). (n =5 individuals per group. Scale bars=50 μm. For all statistical results, the data are shown as the means ± SD. **$p< 0.01$).

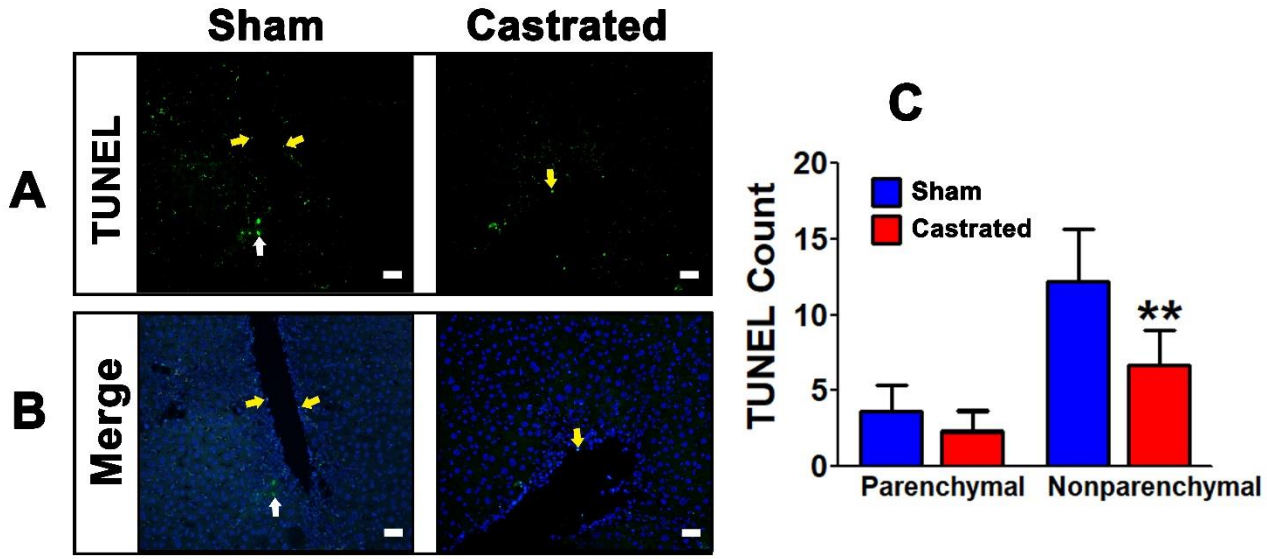

Figure S4. Castration of male reduces noparenchynal cell apoptosis in CCl_4 treated mice
(A-B) TUNEL analysis of sham-operation and castrated mice liver sections (A) and merged with DAPI stain (B). The white arrows highlight TUNEL positive parenchymal cells and the yellow arrows highlight TUNEL positive nonparenchymal cells. (C) Quantitative analysis of TUNEL positive cells in liver sections of sham-operation and castrated mice (t test, Parenchymal p=0.154; Nonparenchymal p=0.004). (n=5 individuals per group. For all statistical results, the data are shown as the means ± SD. **p< 0.01. Scale bars=50 μm.).

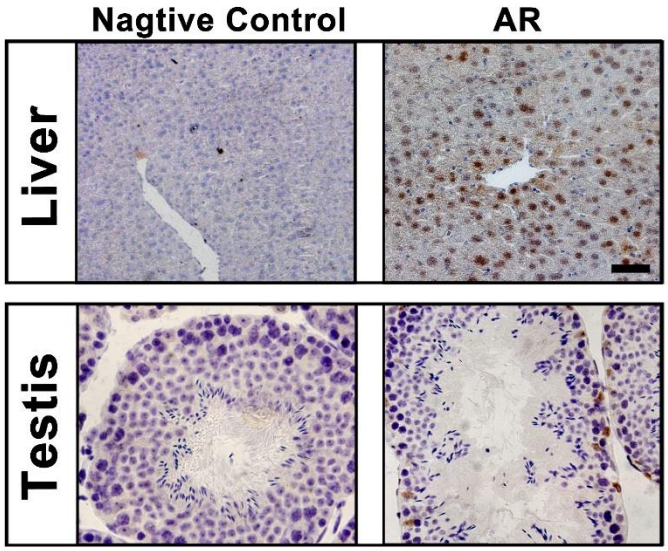

Figure S5. Distribution of androgen receptor (AR) in adult male mice liver and testis.
AR expression was mainly detected in the nucleus of liver parenchymal cells (brown stain) of adult male mouse by immunohistochemical (IHC). The negative controls were performed according to standard IHC procedure, except that AR specific primary antibodies were not used. Mouse testis sections were used as the positive control.

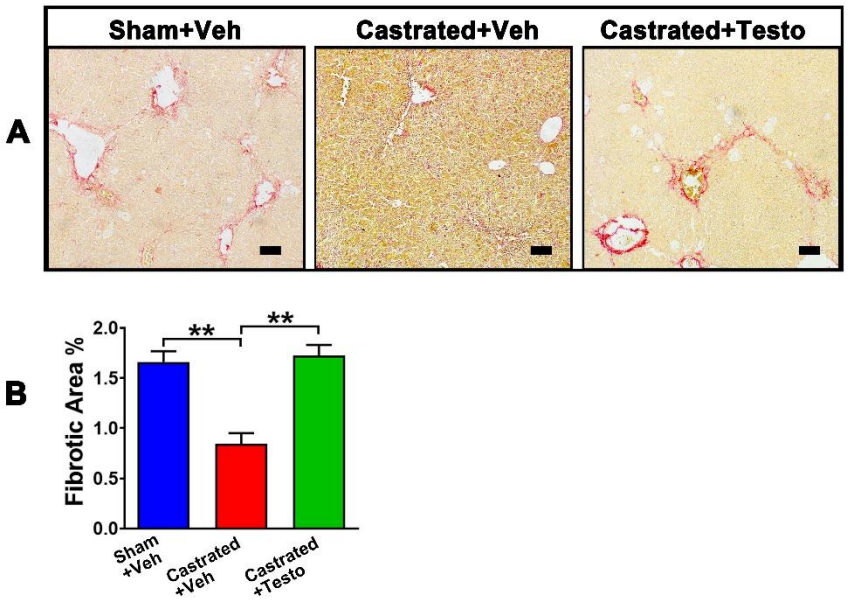

Figure S6 Testosterone aggravates liver fibrosis in CCl_4 treated mice.

Castrated mice received a treatment of testosterone at the dosage of 10mg/kg body weight (Castrated+Testo) or equal volume of vehicle control (Castrated+Veh), and sham-operated male mice received equal volume of vehicle control (Sham+Veh), once every two days for two weeks based on CCl4 induced liver injury mouse model. (A) Sirius red staining of mice liver sections. (B) Quantification of Sirius red area in liver sections. (n = 5 individuals per group. Statistical analysis was performed using one-way analysis of variance (ANOVA) and the group means were compared by Tukey's multiple comparison method. All data are shown as the means ± SD. ** $p < 0.01$. Scale bars=50 μm).

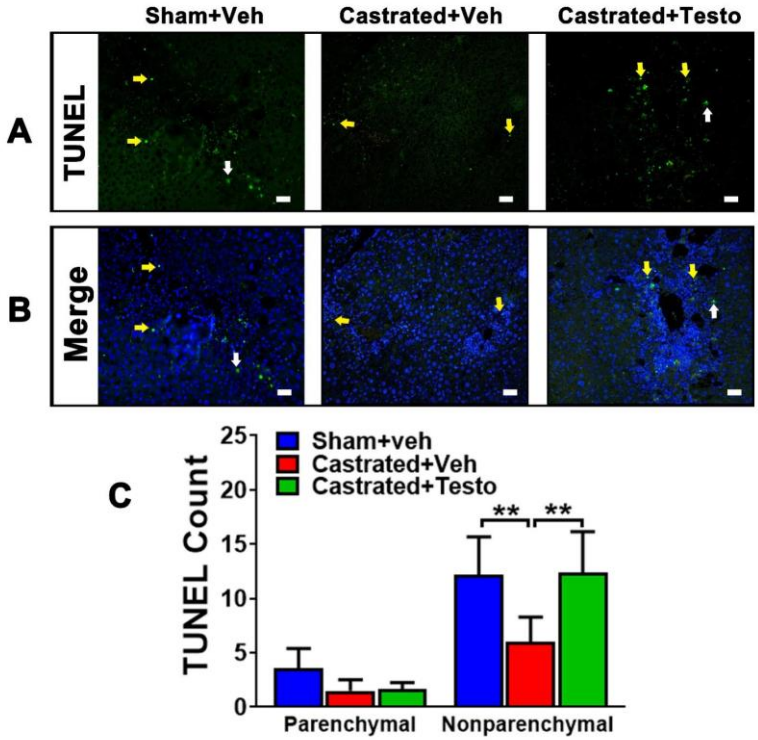

Figure S7. Testosterone regulates liver cell apoptosis in CCl_4 treated mice

(A-B) TUNEL staining of mice liver sections. The white arrows highlight TUNEL positive parenchymal cells, and the yellow arrows highlight TUNEL positive nonparenchymal cells. (C) Quantitative analysis of TUNEL positive cells in liver sections. (n =5 individuals per group. Statistical analysis was performed using one-way ANOVA and the group means were compared by Tukey's multiple comparison method. All data are shown as the means ± SD. ** $p< 0.01$. Scale bars=50 μm).

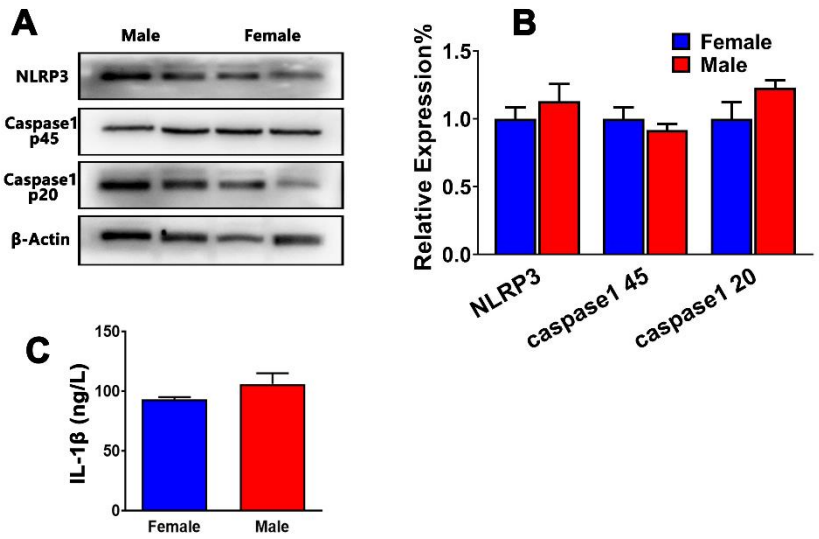

Figure S8 Activation of NLRP3 inflammasome in the liver does not differ between male and female mice. (A) Western blotting analysis of the production of NLRP3, caspase1 p45, and caspase1 p20 in the liver of male and female mice. (B) Quantification of the expression levels of NLRP3, caspase1 p45, and caspase1 p20 in the liver of male and female mice. (t test, NLRP3 p=0.188, Caspase1 p45, p=0.252, Caspase1 p20, p=0.257). (C) IL-1β production in the serum of male and female mice (t test, p=0.178). (n =4 individuals per group. For all statistical results, the data are shown as the means ± SD.).

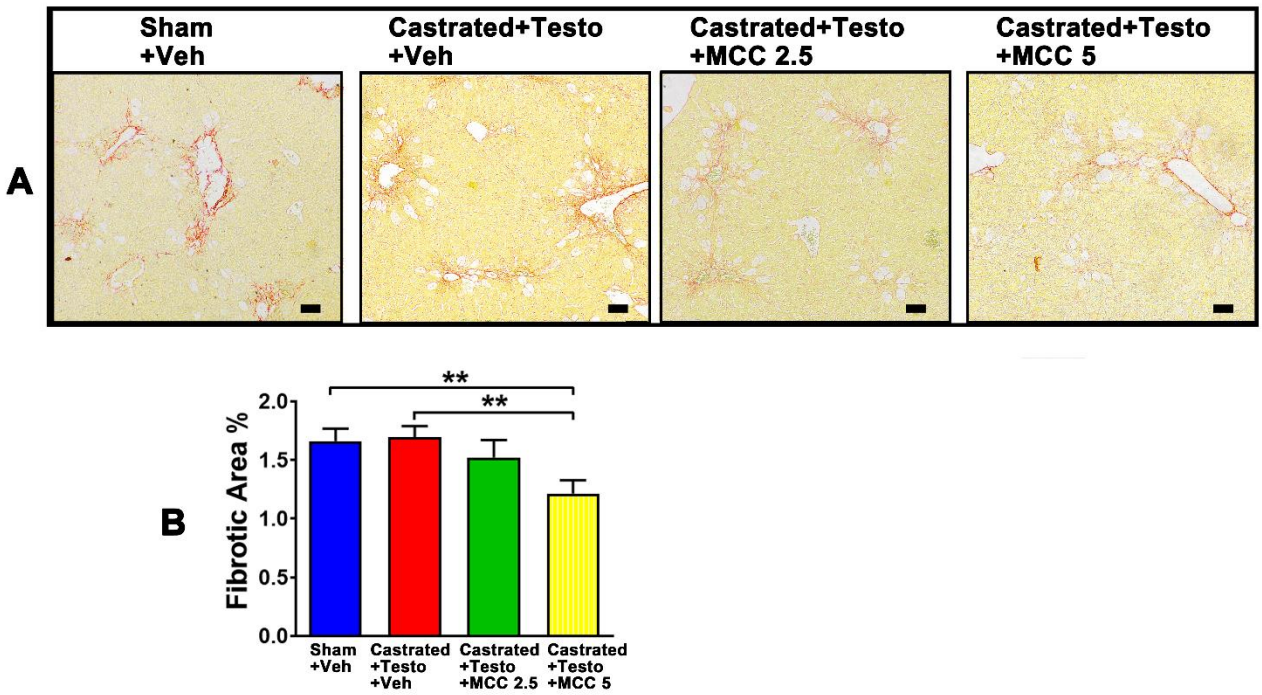

Figure S9. MCC950 reduces liver fibrosis in testosterone and CCl_4 treated mice.

Testosterone treated castrated male mice received a treatment of MCC950 at the dosage of 2.5mg/kg (Castrated+Testo+MCC 2.5) or 5 mg/kg (Castrated+Testo+MCC 5) body weight or equal volume of saline control (Castrated+Testo+Veh) once a day for two weeks based on CCl4 induced liver injury. And sham-operated male mice received equal volume of saline (Sham+Veh) once a day for two weeks based on CCl4 induced liver injury. (A) Sirius red staining of liver sections. (B) Quantification of Sirius Red area in liver sections. (n =4 individuals per group. Statistical analysis was performed using one-way ANOVA and the group means were compared by Tukey's multiple comparison method. All data are shown as the means ± SD. **p< 0.01. Scale bars=50 μm).