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Supplemental Information

MicroRNA 223 3p Negatively Regulates the NLRP3 Inflammasome in Acute and Chronic Liver Injury

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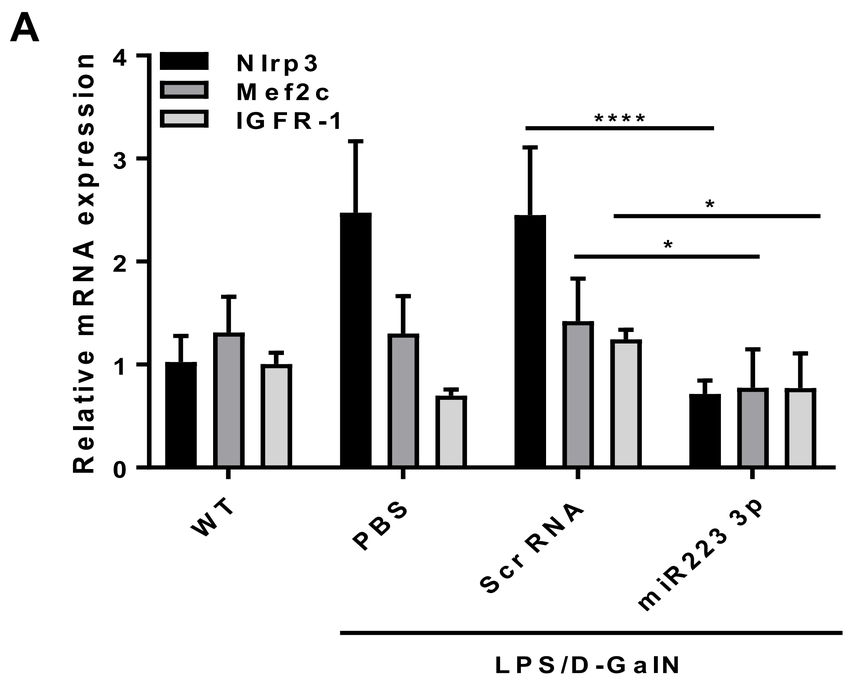
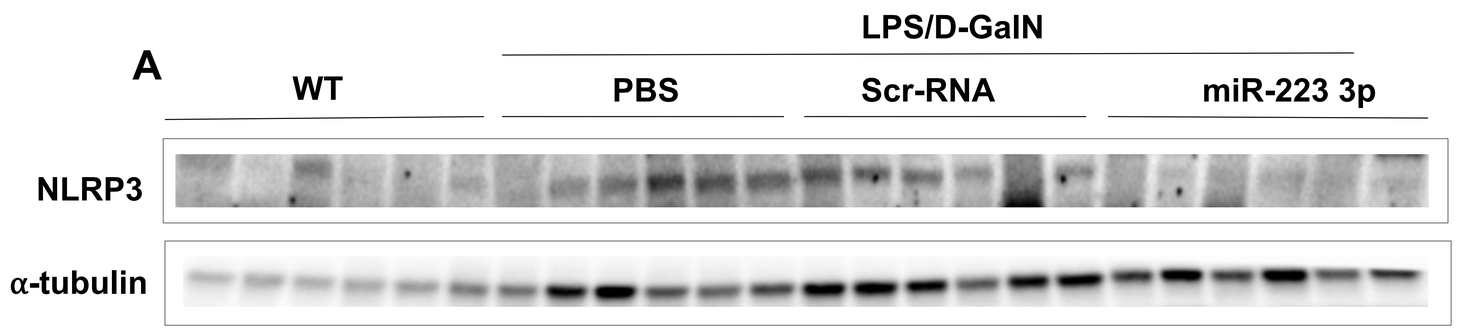


Fig. S1 (A) Relative mRNA expression of *Nlrp3*, *Mef2c* and *Igfr1* measured by qRT-PCR and normalized by *B2m.* **P*<0.05, *****P*<0.0001, two-way ANOVA. Results show means ± SD, n=7-10/group



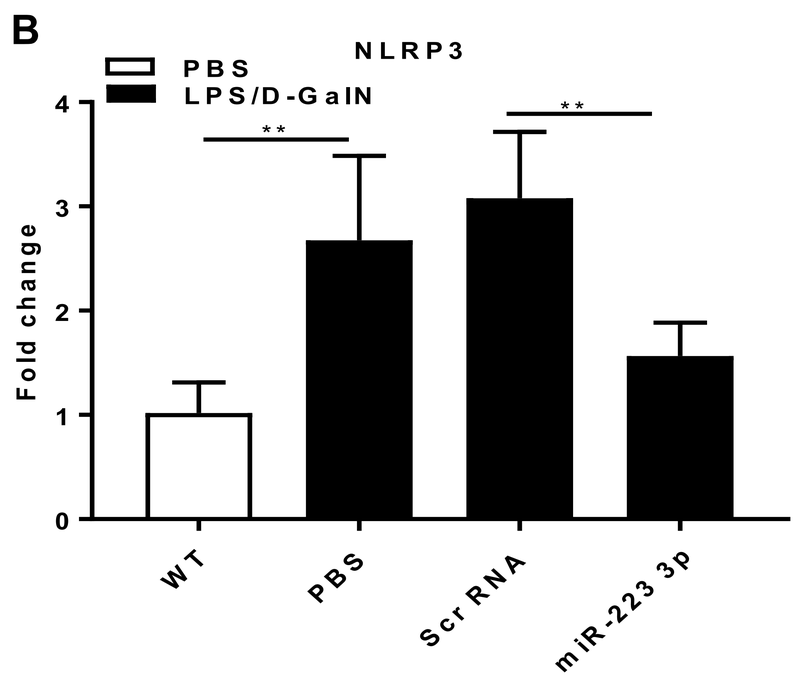


Fig. S2 (A) Western blots of NLRP3 and α-tubulin proteins (original blots shown in Supplementary Figure 3). (**B**) Fold change of intensity of protein bands of NLRP3 shown in **Fig S2A**. Normalization by the housekeeping protein α-tubulin. ***P*<0.01; one-way ANOVA. Data are represented as means ± SD, n=6/group.

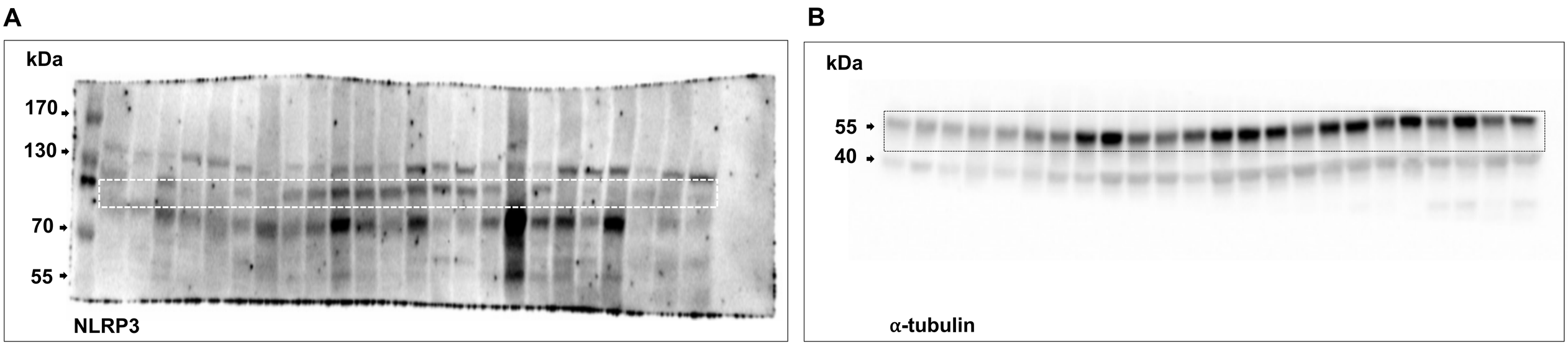
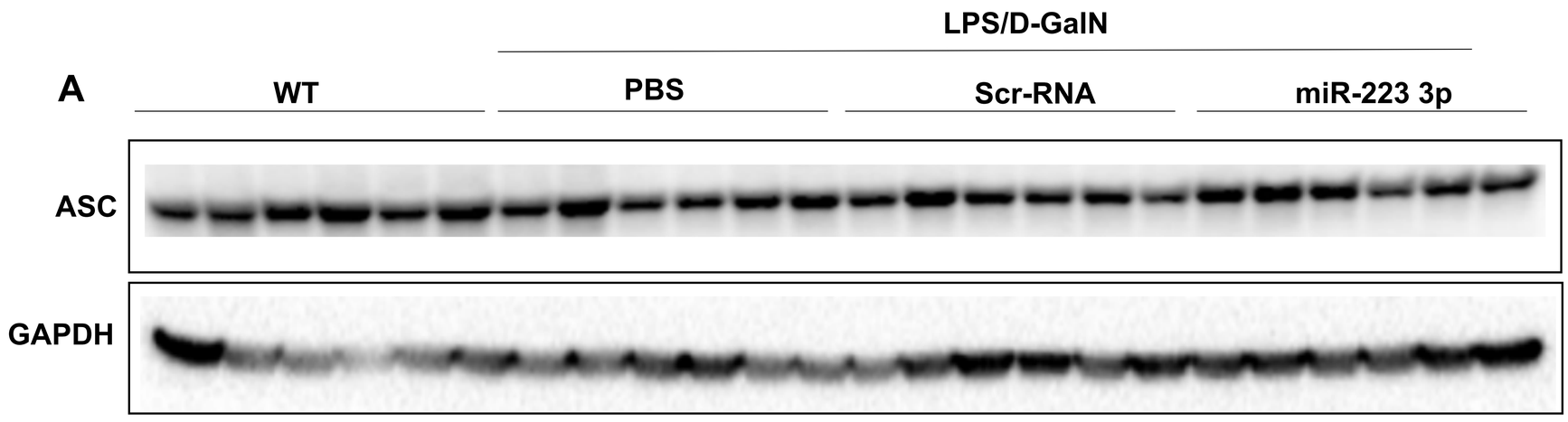


Fig. S2 (A-F) Uncropped protein blots of NLRP3 and α-tubulin, shown in **Supplementary Figure 2A** and, GAPDH and cleaved IL-1ß displayed in **Fig. 2**. Specific protein bands are marked with white or black, slashed quadrants and are selected at predicted molecular weights (kDa) on the left of the blot.



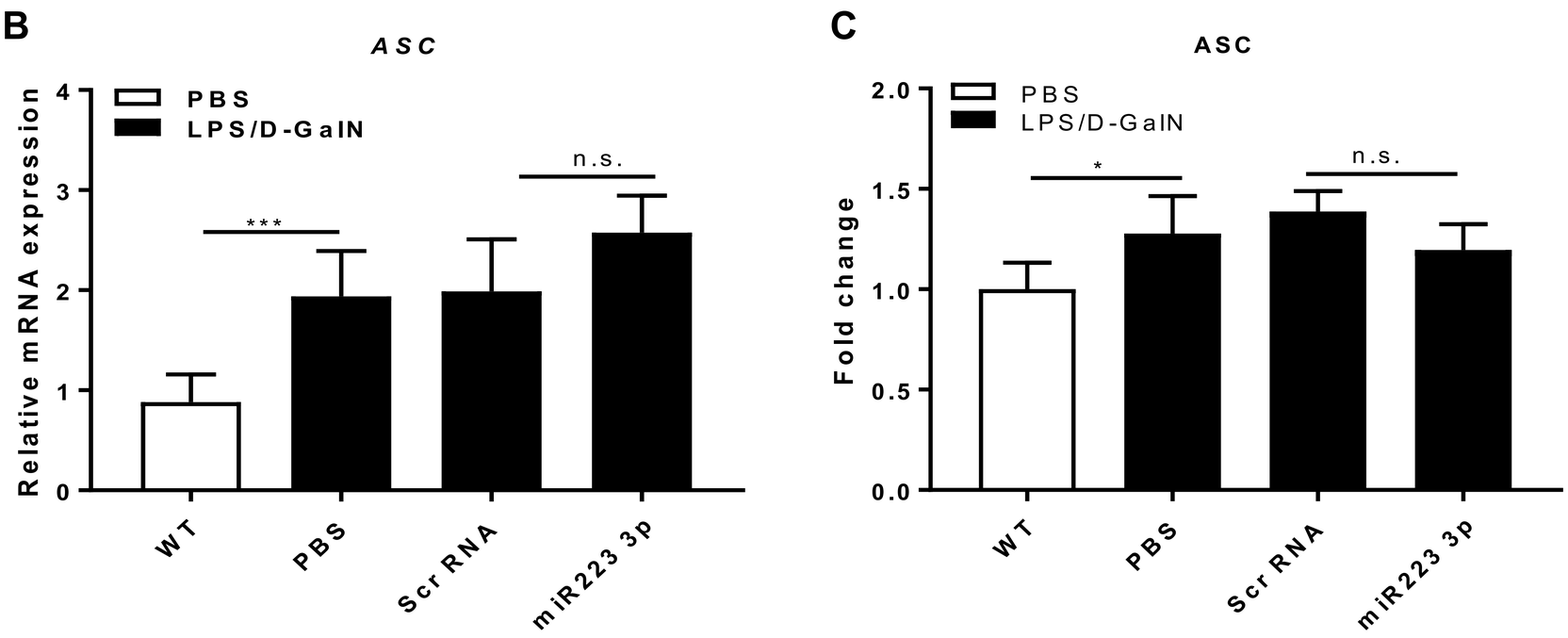


Fig. S3 (A) Representative western blots of ASC and GAPDH protein bands (uncropped blots are shown in Supplementary Figure 4) from mouse livers of **experiment in Figure 1*A* (B)** Relative mRNA expression of *Asc* measured by qRT-PCR and normalized by the housekeeping gene *Gapdh.* (**C**) Fold change of ASC protein levels normalized against GAPDH. **P*<0.05, ****P*<0.001; n.s., not significant; one-way ANOVA. Results show means ± SD, n=7-10/group.

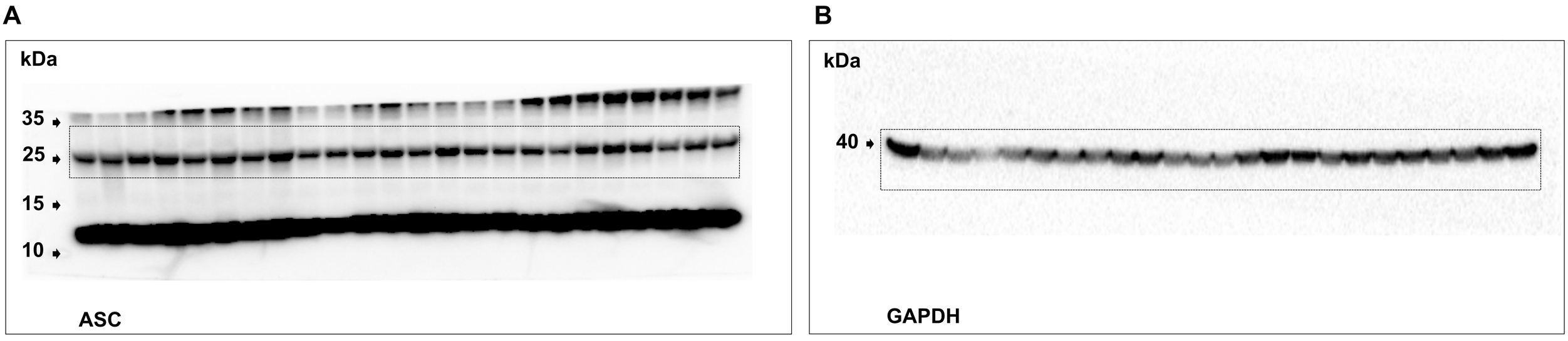
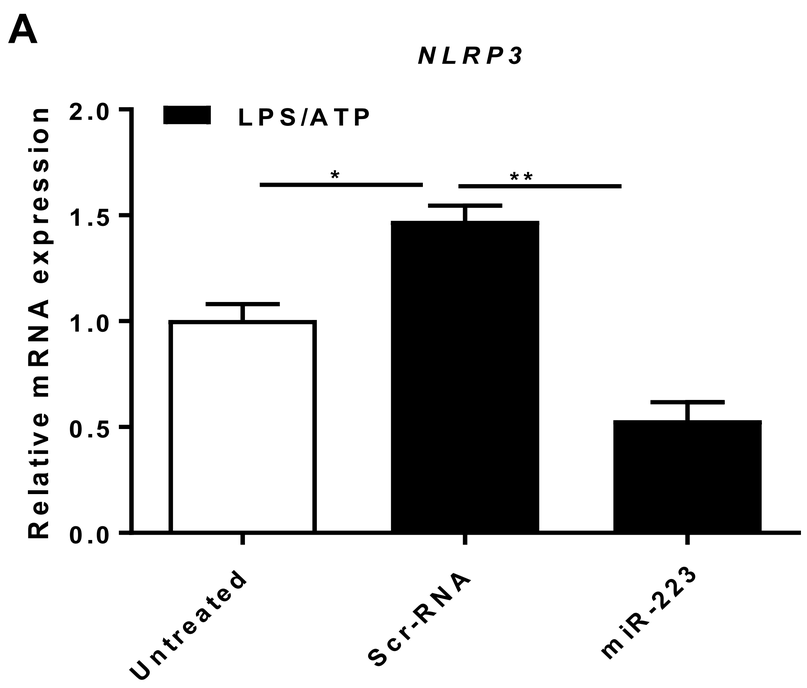
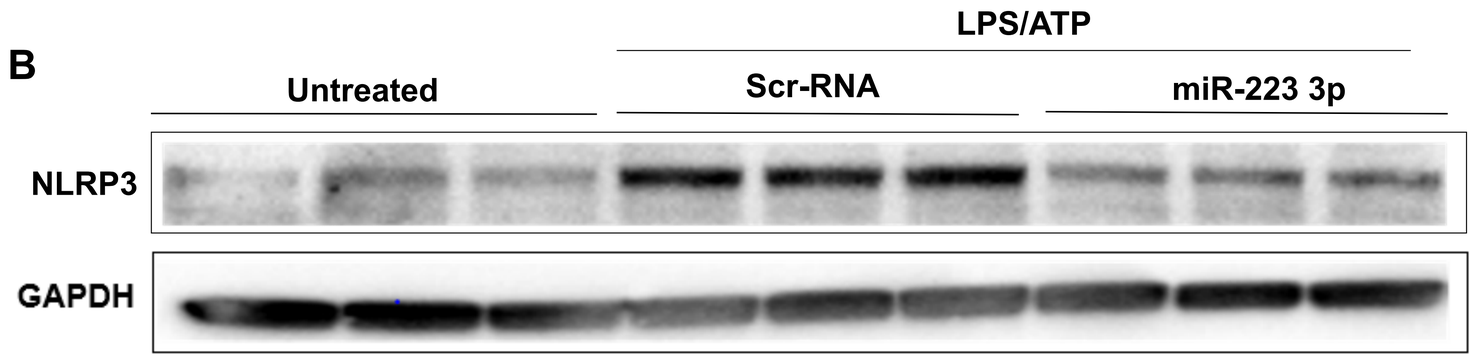
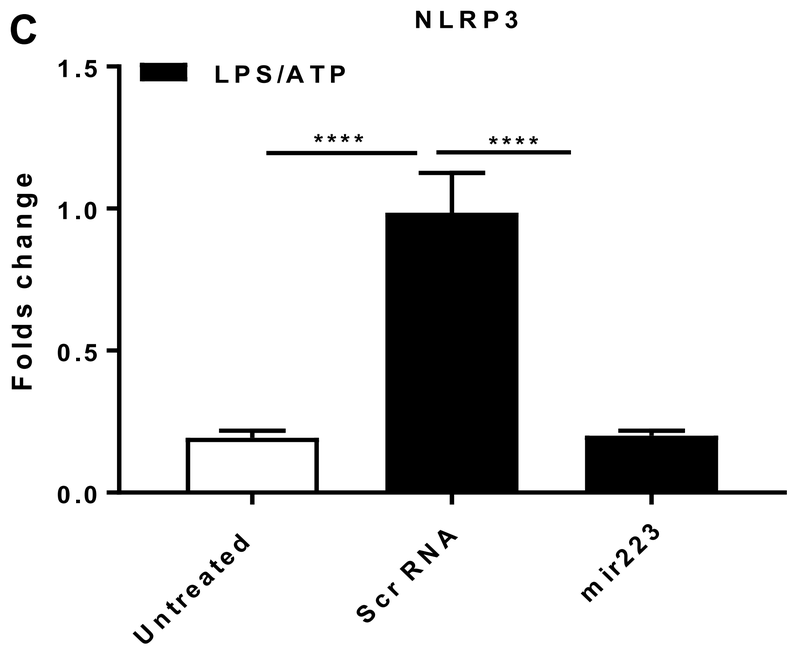
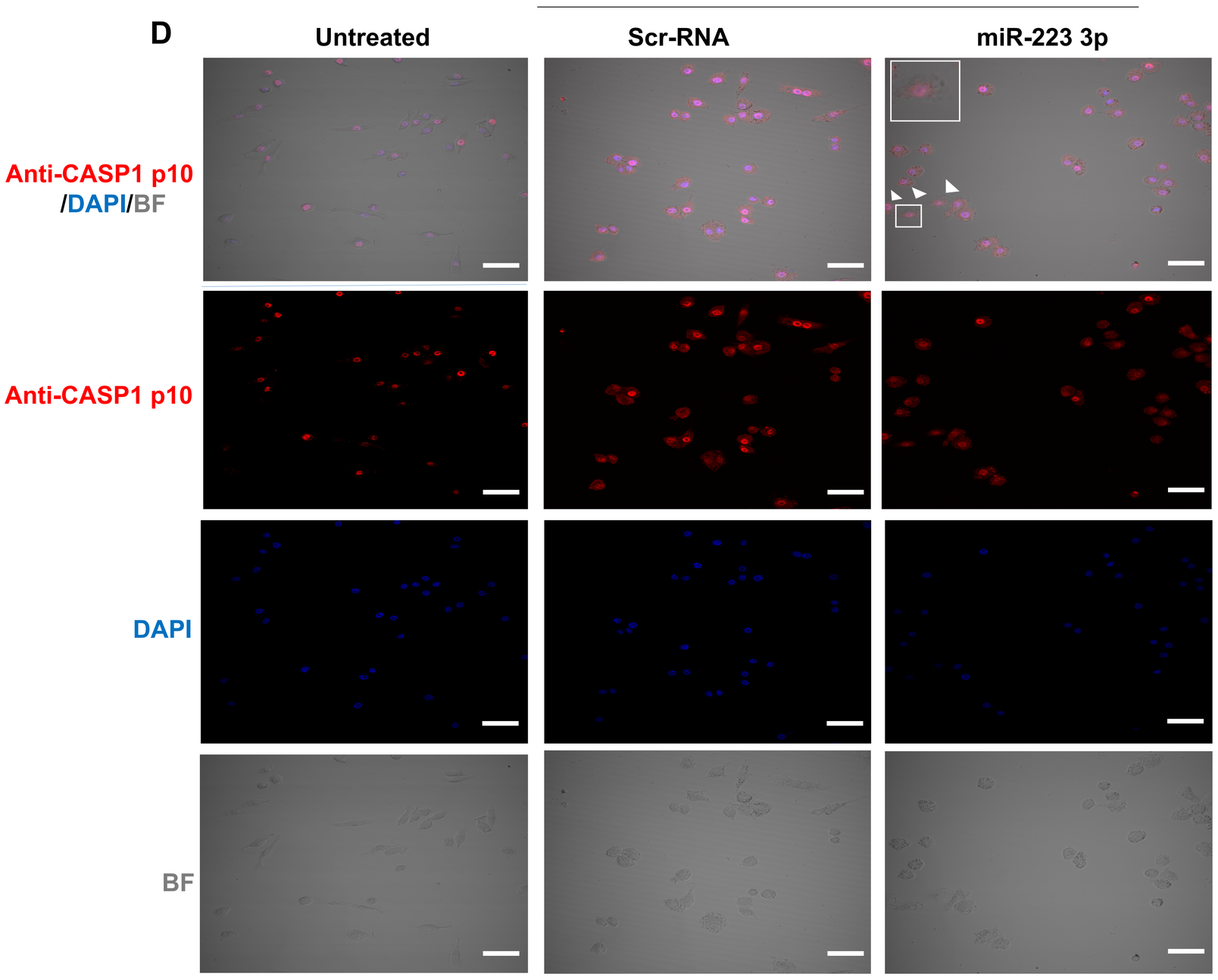


Fig. S4 (A-B) Uncropped protein blots of ASC and GAPDH displayed in **Fig. S3**. Specific protein bands are marked with white or black, slashed quadrants and are selected at predicted molecular weights (kDa) on the left of the blot.









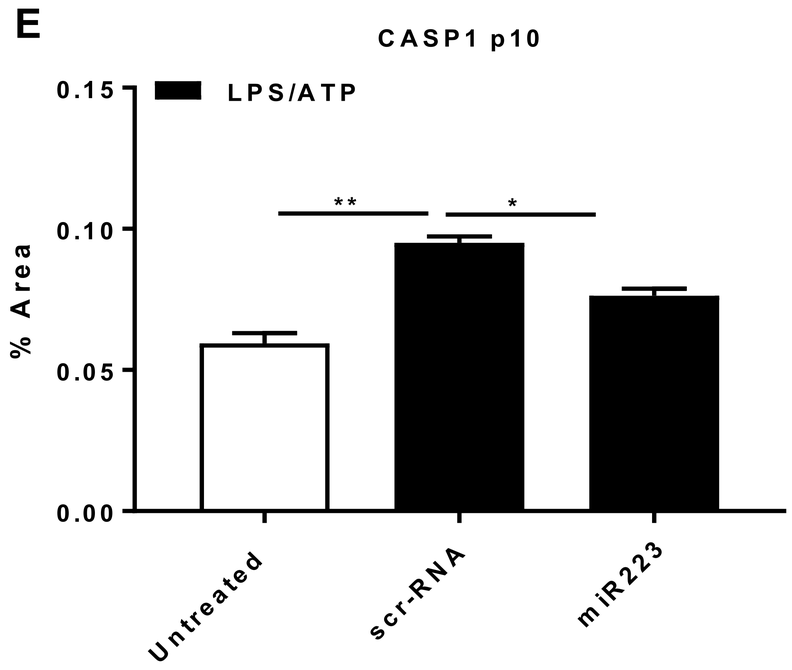


Fig. S5 (A) Relative *Nlrp3* mRNA expression assessed by qRT-PCR and normalized by the housekeeping gene *Gapdh* in J744.2 macrophages transfected with miR-223 3p or Scr-RNA control and pre-treated or untreated with LPS/ATP (**B**) Representative Western blots of NLRP3 and GAPDH protein bands from experiment described in **A** (original blots are shown in **Supplementary Figure 6**). (**C-E**) Fold change of NLRP3 levels normalized by the housekeeping protein GAPDH. (**F**) Representative microphotographs of immunofluorescence staining in cells from experiment in **A**. Alexa Fluor 598-Anti-caspase 1 p10 (active form) antivody shown in red, DAPI in blue represents nuclei and bright filter (BF) shows cellular body in grey. Representative cells with reduced cytosolic signal for anti-caspase 1 p10 are indicated with white arrows and amplified in a slashed upper quadrant. Scale bars=100 μm. (**G**) Percentage of fluorescence area normalized by total number of cells per field. **P*<0.05; ***P*<0.01; *****P*<0.0001; n.s., not significant, one-way ANOVA. Results show means ± SD, n=3/group

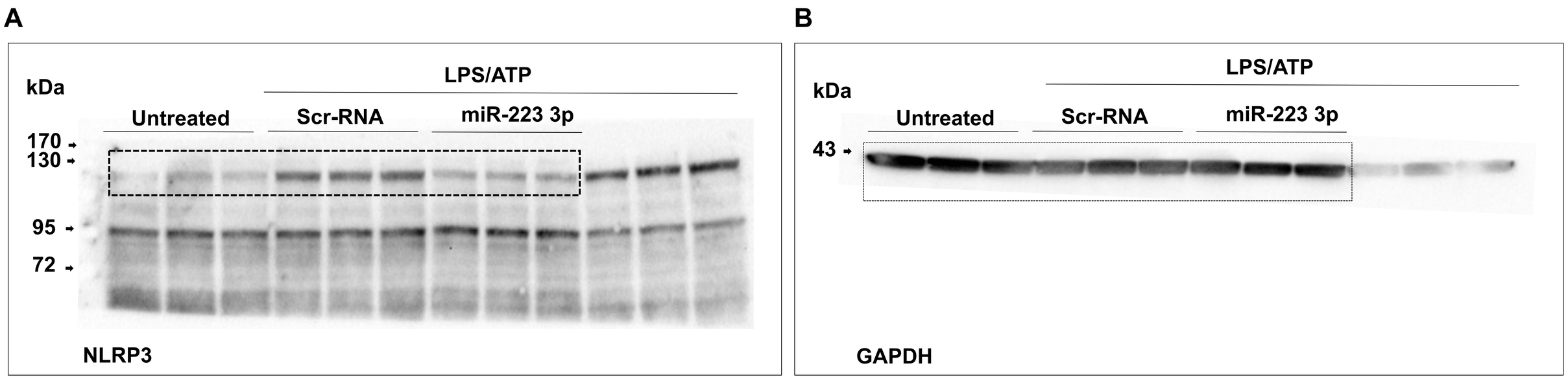


Fig. S6 (A-D) Uncropped protein blots of NLRP3 and GAPDH shown in **Fig. S5**. Specific protein bands are marked with white or black, slashed quadrants and are selected at predicted molecular weights (kDa) on the left.