Atractylenolide I Prevents Acute Liver Failure in Mouse by Regulating M1 Macrophage Polarization

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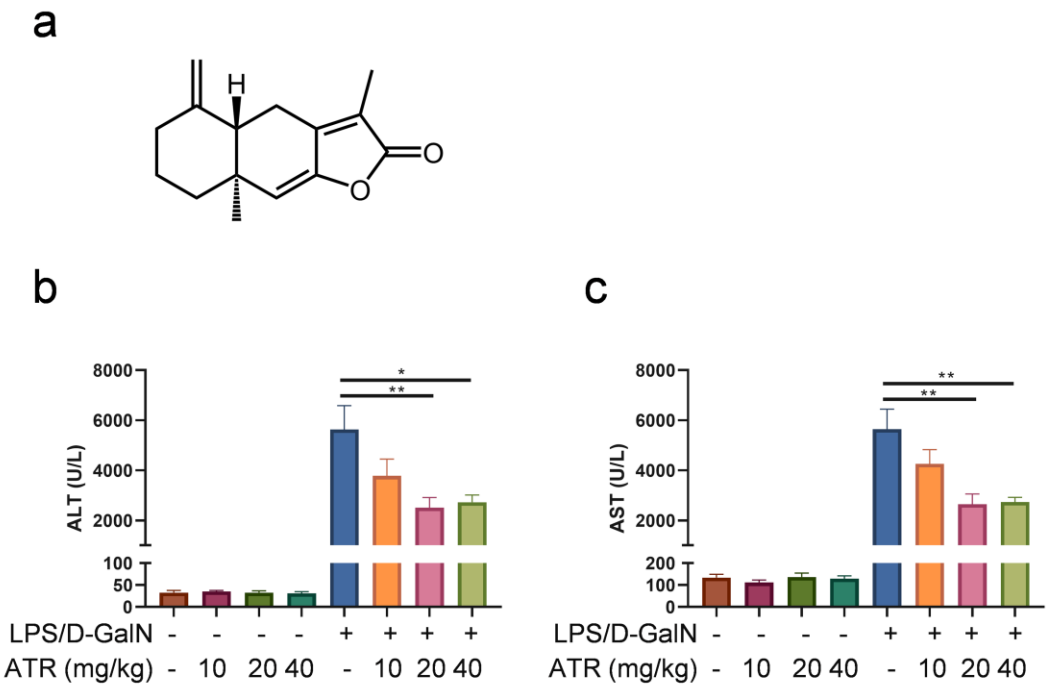
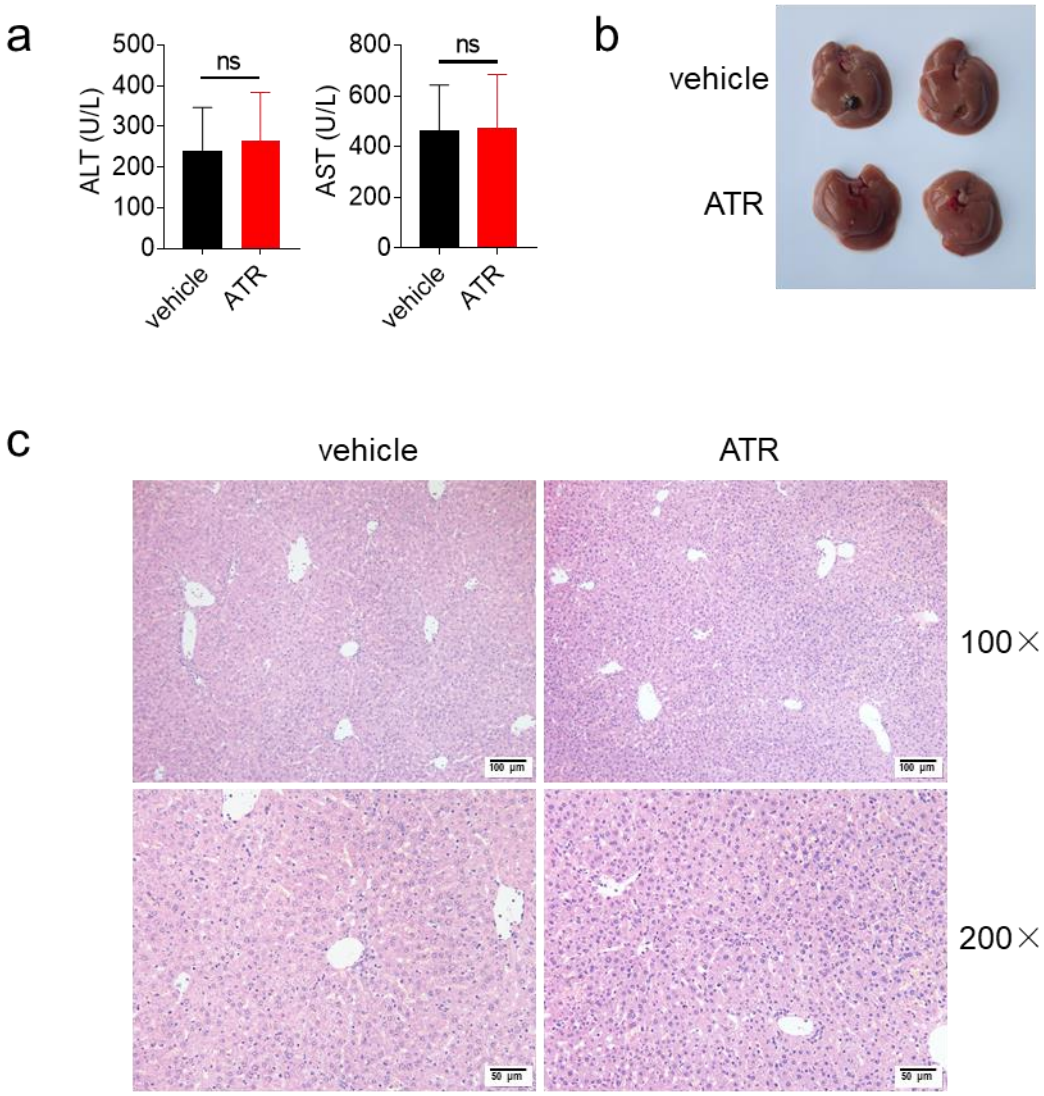
Supplementary Fig. S1 online

The effects of different concentrations of ATR-I on LPS/D-GalN-Induced ALF. Mice were intraperitoneally injected with ATR-I (10, 20, 40 mg/kg body weight) 3 h before being intraperitoneally adminstrated with LPS (10 μg/kg)/D-GalN (250 mg/kg). Twelve hours after LPS/D-GalN injection, the serum were collected. (a) Chemical structure of ATR-I. (b) Serum ALT levels. (c) Serum AST levels. Data represent means ± SD (n = 5). **P* < 0.05, ***P* < 0.01



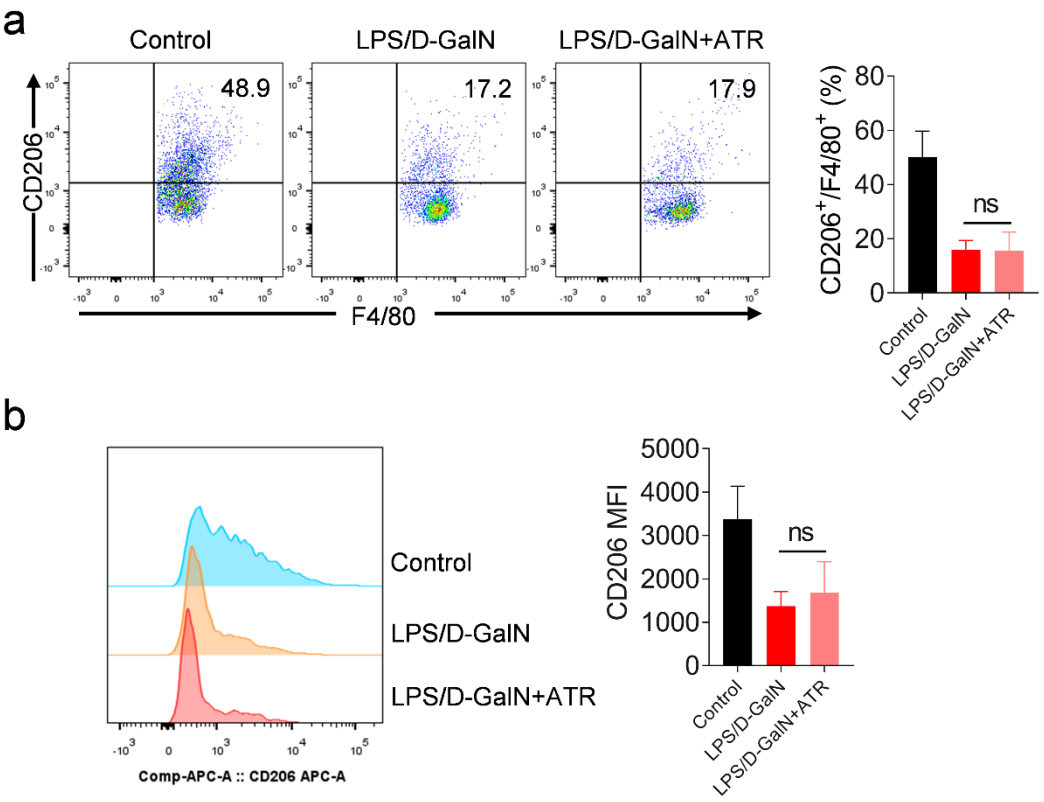
Supplementary Fig. S2 online

ATR-I does not elicit negative side effects in mice. Mice were intraperitoneally injected with ATR-I (20 mg/kg). After 15 h, the liver tissue and serum were collected. (**a)** Serum ALT and AST levels in mice (n=5). (**b)** Liver appearance. (**c)** Liver sections stained with H&E at original magnifications of 100× and 200×



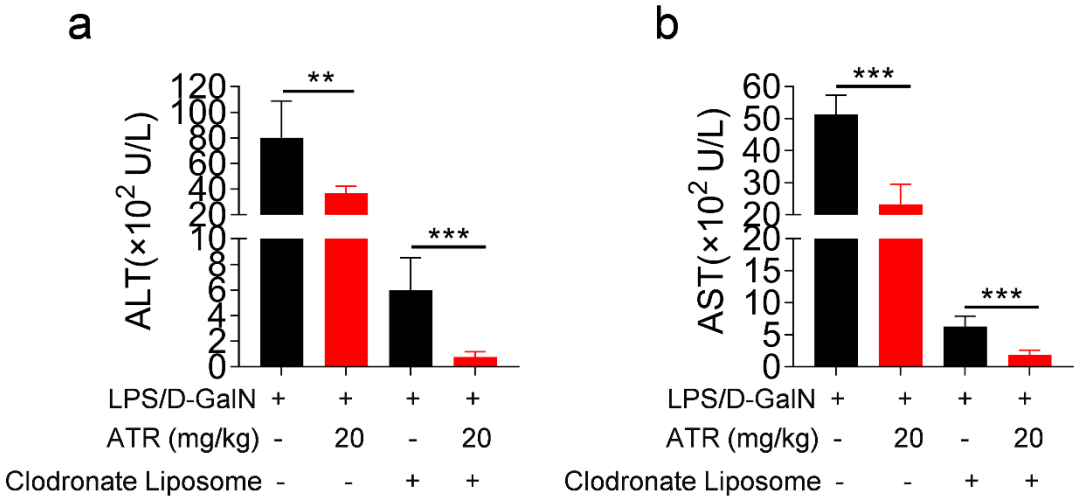
Supplementary Fig. S3 online

ATR-I does not impact M2 macrophage activation *in vivo*. Mice were intraperitoneally injected with ATR-I (20 mg/kg) and 3 h later were injected intraperitoneally with LPS (10 μg/kg)/D-GalN (250 mg/kg). After 12 h, the liver tissues were collected. (**a)** Ratio of M2 macrophages in mouse HMNCs ($CD206^+F4/80^+$) (n=5). (**b)** CD206 mean fluorescence intensity (n=5). Data are presented as the mean ± SD, ns, not significant



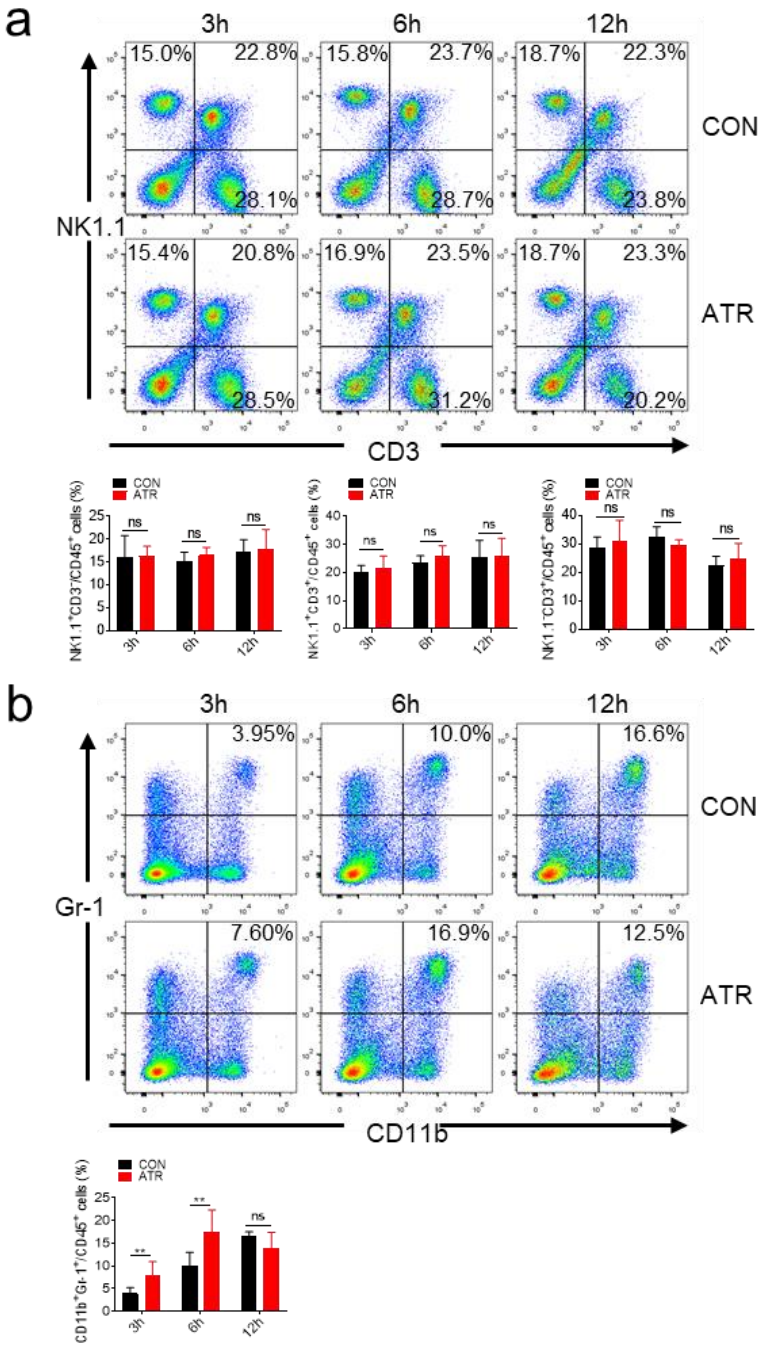
Supplementary Fig. S4 online

The hepatoprotective effects of ATR-I is partially related to M1 macrophage polarization. Liver macrophages were depleted with clodronate liposomes (50 μL/10 g of body weight) by intravenous injection in accordance with the guidelines of the manufacturer. Twenty-four hours later, the mice were treated with ATR-I (20 mg/kg body weight) and LPS (10 μg/kg body weight)/D-GalN (250 mg/kg body weight). Serum (**a**) ALT and (**b**) AST levels in mice (n=5). Data are presented as the mean ± SD. $^{**}p < 0.01$, $^{***}p < 0.001$



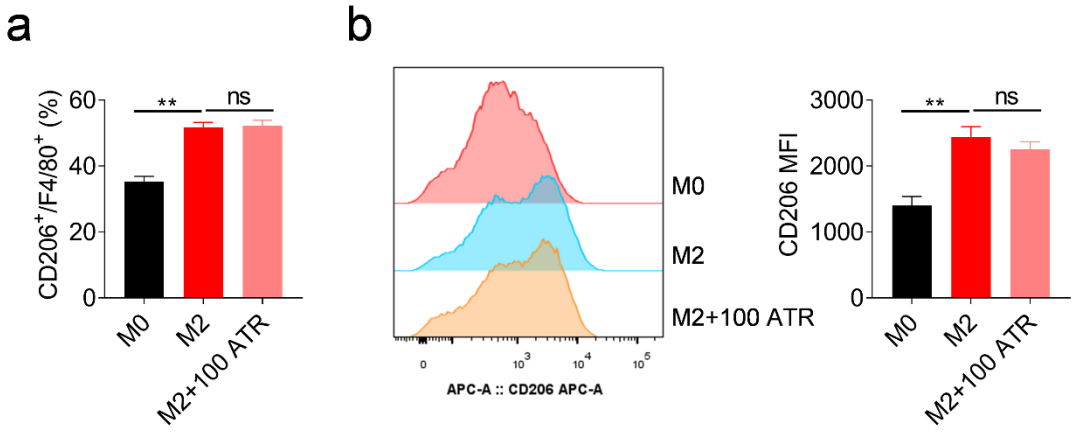
Supplementary Fig. S5 online

Effects of ATR-I on the proportion of NK, NKT, T cells and MDSCs in mice livers. Mice were intraperitoneally injected with ATR-I (20 mg/kg) and 3 h later were injected intraperitoneally with LPS (10 μg/kg)/D-GalN (250 mg/kg). Liver tissues were collected at the indicated time points after LPS/D-GalN injection. (**a**) NKT ($CD3^+NK1.1^+$), NK ($CD3^-NK1.1^+$), and T ($CD3^+NK1.1^-$) cell ratios in mouse hepatic mononuclear cells (HMNCs) (n=5). (**b**) MDSCs ($CD11b^+Gr\text{-}1^+$) ratios in mouse HMNCs (n=5). Data are presented as the mean ± SD. $^{**}p < 0.01$, ns, not significant



Supplementary Fig. S6 online

ATR-I does not affect M2 macrophage activation *in vitro*. BMDMs were treated with ATR-I (100 μM) for 3 h and then supplemented with IL-4 (10 ng/mL) and IL-13 (10 ng/mL) for 24 h. (**a)** Ratio of M2 macrophages after ATR-I treatment (n=5). (**b**) CD206 mean fluorescence intensity after ATR-I treatment (n=5). Data are presented as the mean ± SD. $^{**}p < 0.01$, ns, not significant



Supplementary Fig. S7 online

The inhibitory effects of ATR-I on M1 macrophage polarization is partially dependent on the MAPK signaling pathway. BMDMs were treated with ATR-I (100 μM) and Anisomycin (10 μg/mL) for 3 h and then supplemented with LPS (100 ng/mL) and IFN-γ (20 ng/mL) for 24 h. **(a)** Ratio of M1 macrophages ($CD86^+CD11B^+$) (n=5). **(b)** CD86 mean fluorescence intensity (n=5). Data are presented as the mean ± SD. **p* < 0.05, ****p* < 0.001