In this study, we learned whether the absence of Sprouty-related EVH1-domain-containing protein 2 (Spred2), a negative regulator of the Ras/Raf/ERK/MAPK pathway, influences ALI induced by D-galactosamine (D-GalN) and lipopolysaccharide (LPS). Compared to wild-type mice, Spred2-/- mice developed exacerbated liver injury represented by enhanced hepatocyte damage and inflammation. Enhanced ERK activation was observed in Spred2-/- livers, and the MEK/ERK inhibitor U0126 ameliorated ALI. Hepatic tumour necrosis factor (TNF) and interleukin (IL)-1β levels were increased in Spred2-/- livers, and the neutralization of TNFα dramatically ameliorated ALI, which was associated with decreased levels of endogenous TNFα and IL-1β. When mice were challenged with D-GalN and TNFα, much severer ALI was observed in Spred2-/- mice with significant increases in endogenous TNFα and IL-1β in the livers. Immunohistochemically, Kupffer cells were found to produce TNFα, and isolated Kupffer cells from Spred2-/- mice produced significantly higher levels of TNFα than those from wild-type mice after LPS stimulation, which was significantly decreased by U0126. These results suggest that Spred2 negatively regulates D-GalN/LPS-induced ALI under the control of TNFα in Kupffer cells. Spred2 may present a therapeutic target for the treatment of ALI.