Trans-Chalcone attenuates pain and inflammation in experimental acute gout arthritis in mice

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Running title: *Trans*-chalcone attenuates gout arthritis

SUPPLEMENTARY DATA

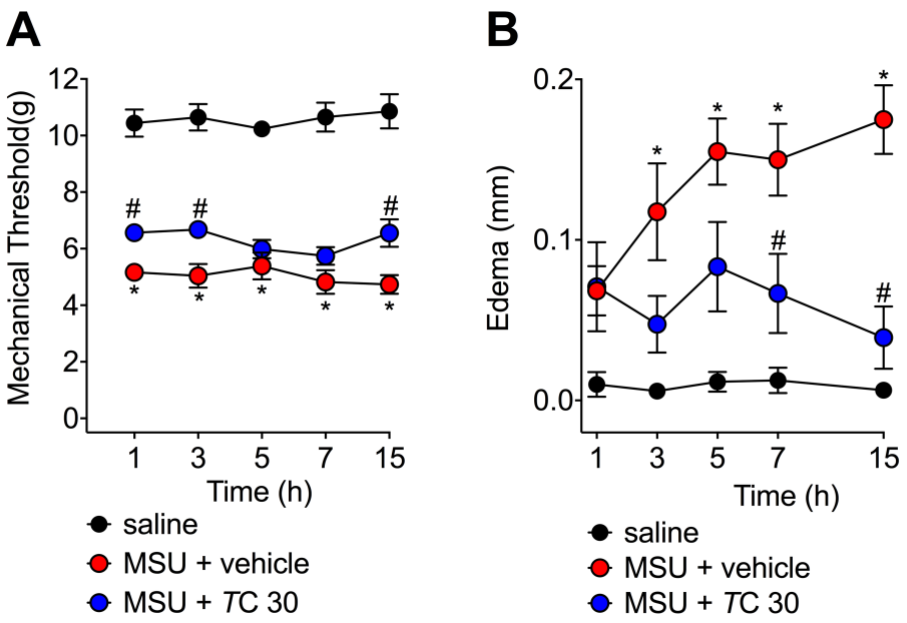

Figure S1. Effect of trans-chalcone post-treatment in MSU-induced mechanical hyperalgesia and edema. Mice were treated *Trans*-Chalcone (*T*C, 30 mg/kg, p.o., 100 µl) or vehicle (Tween 80 20% plus saline) 30 minutes after MSU (100 µg/10 µl/knee) or saline stimulus in the femur-tibial joint of swiss mice. (**A**) Mechanical hyperalgesia and (**B**) edema were evaluated 1, 3, 5, 7, and 15h after MSU injection. Results are expressed as mean ± SEM, data represent a total of 12 mice per group that were obtained in two independent experiment with 6 mice per experiment. (*$p < 0.05$ vs. control group; #$p < 0.05$ vs. vehicle group, two-way ANOVA followed by Tukey's post-test).

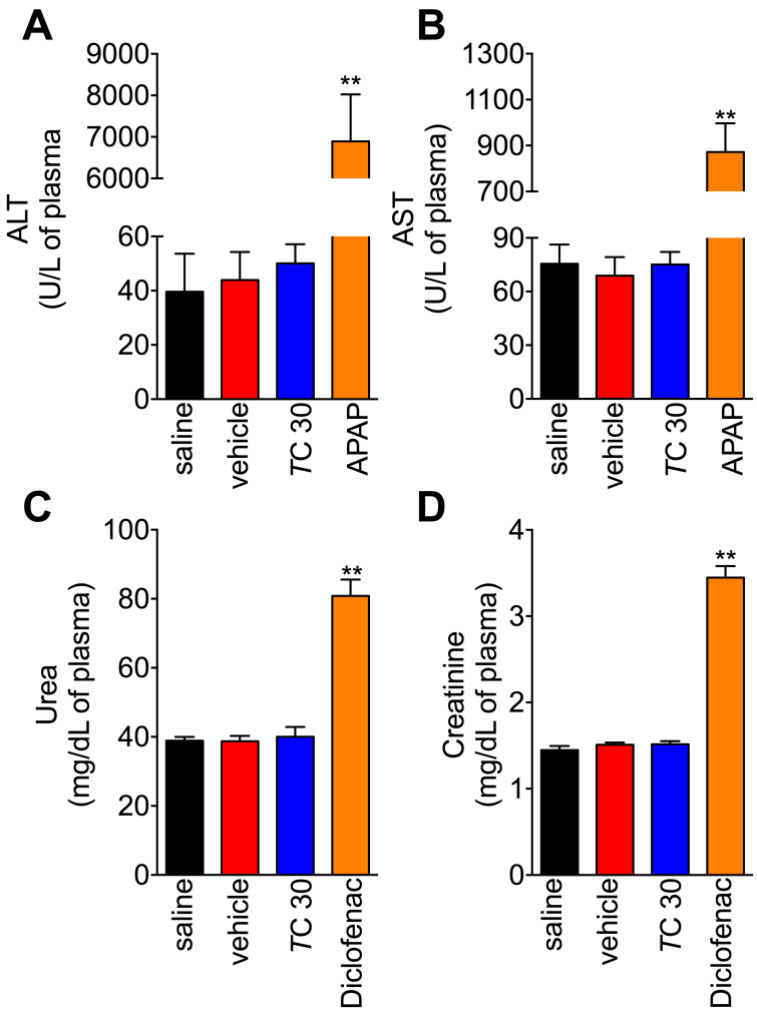

Figure S2. *Trans*-chalcone does not induce kidney or liver injury. Blood was collected 15.5h after treatment with *trans*-chalcone (*T*C, 30 mg/kg, p.o.) or vehicle (Tween 80 20% plus saline) to assess: (**A**) alanine transaminase (ALT), (**B**) aspartate aminotransferase (AST) levels in plasma samples. Acetaminophen stimulus (APAP, 650 mg/kg, p.o.) was used as a control drug for liver injury and samples were collected after 10h after stimulus. (**C**) Urea and (**D**) creatinine levels in plasma samples. Diclofenac stimulus (200 mg/kg, p.o) was used as a control drug for kidney injury and samples were collected 24h after stimulus. Results are expressed as mean ± SEM, data represent a total of 12 mice per group that were obtained in two independent experiment with 6 mice per experiment. (**p < 0.05 vs. all groups, one ANOVA followed by Tukey's post-test).

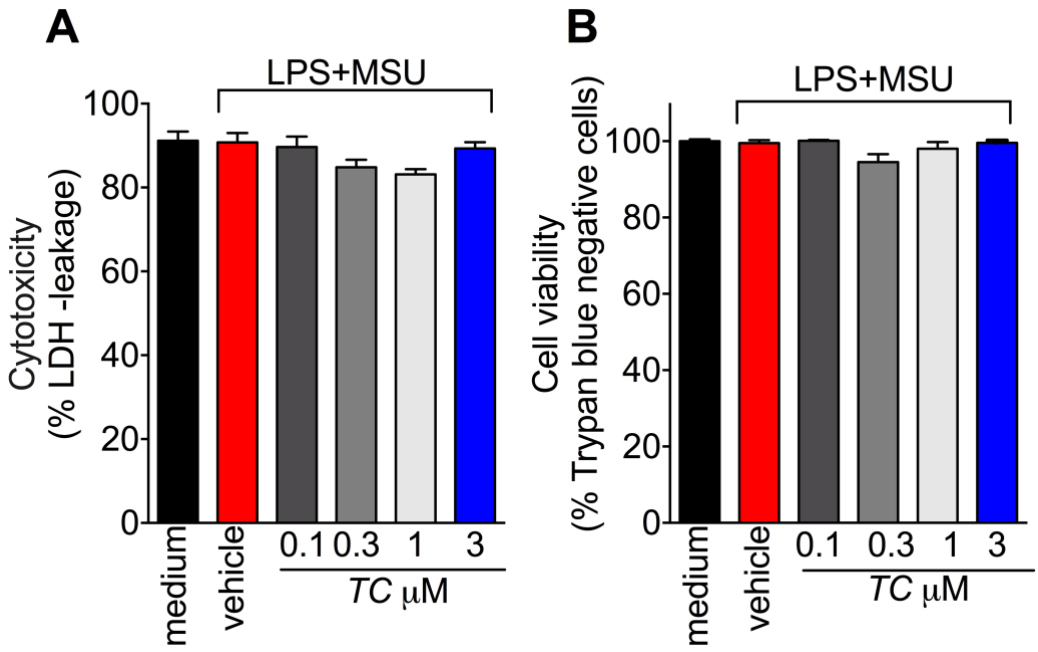

Figure S3. ***Trans*-chalcone does not induce cytotoxicity or alters cell viability.** BMDMs were pre-treated with 0.1-3 μM before 500 ng/mL of LPS (*before* priming) and after 3h were secondarily stimulated with MSU (450 mg/ml, activation). (**A**) Supernatants were collected 5h after MSU stimulation in BMDMs cells to assess LDH levels and (**B**) Trypan Blue assay to determine cell viability. The results were expressed as % of LDH release or dead cells from total cells counted by comparing with the positive control (vehicle group).