iScience, Volume *26*

Supplemental information

Tivantinib alleviates inflammatory diseases by directly targeting NLRP3

Yi Huang, Yun Guo, Yan Zhou, Qian Huang, Yi Ru, Yingli Luo, and Wen Xu

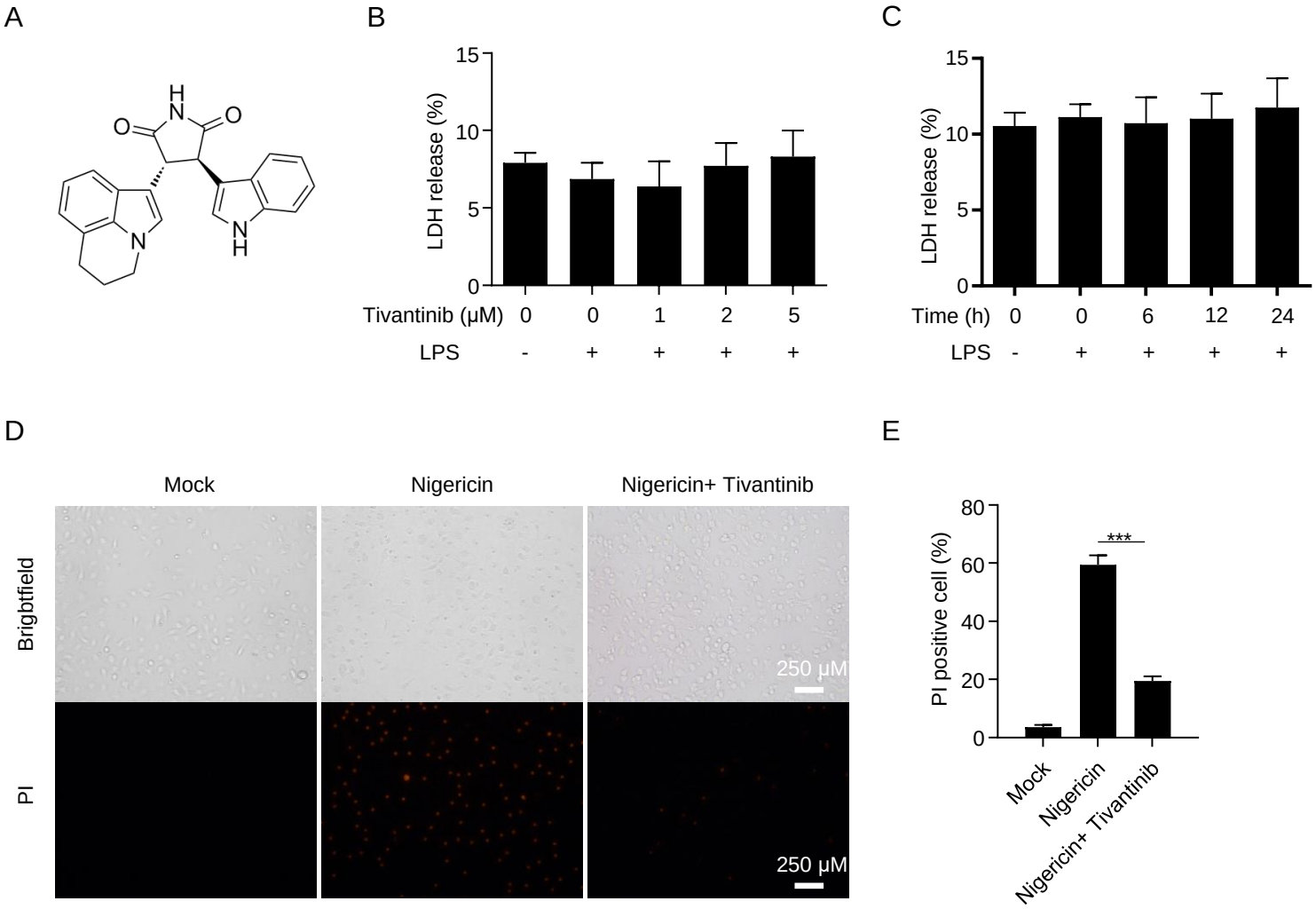

Figure S1. Tivantinib has no cytotoxic and inhibits nigericin-induced pyroptosis, Related to Figure 1. (A) The structure of Tivantinib. (B) LDH release in SN of LPS-primed BMDMs treated with various concentrations of Tivantinib (as labeled). (C) LDH release in SN of LPS-primed BMDMs treated with 5 μM Tivantinib for various times. (D) Images of LPS-primed BMDMs treated with 5 μM Tivantinib in the presence of Propidium Iodide (PI) and then stimulated with 5 μM nigericin for 30 min. (E) The percentage of PI^+ BMDMs treated with 5 μM Tivantinib and then stimulated with 5 μM nigericin. Data represent means ± SEM from four biological duplicates (B, C, E). Statistical analysis was performed using unpaired Student's t-test (E). ****P < 0.001.*

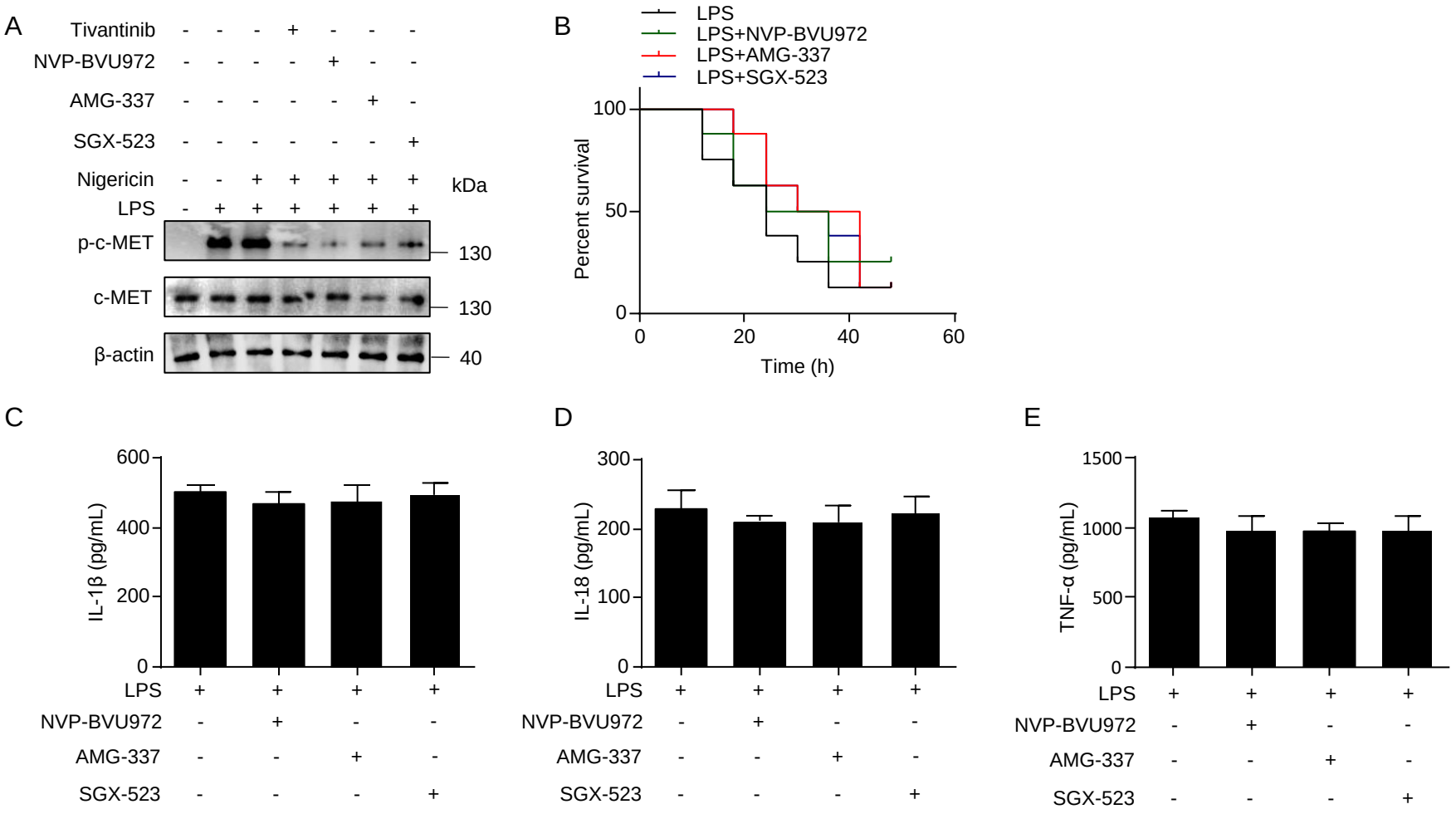

Figure S2. NVP-BVU972, AMG-337 and SGX-523 inhibit c-Met signal activation and had no effect on LPS-induced sepsis, Related to Figure 3 and Figure 5. (A) Western blot analysis of c-Met phosphorylation in Input of LPS-primed BMDMs treated with 5 μM Tivantinib, 1 μM NVP-BVU972, 1 μM AMG-337, 1 μM SGX-523, and then stimulated with 5 μM nigericin. (B) Survival analysis of C57BL/6J mice intraperitoneally injected with LPS (20 mg/kg) and pre-treated with vehicle, NVP-BVU972 (10 mg/kg), AMG-337 (10 mg/kg) or SGX-523 (10 mg/kg). n = 8 biologically independent mice. (C-E) ELISA analysis of IL-1β (C), IL-18 (D) and TNF-α (E) in serum of C57BL/6J mice intraperitoneally injected with LPS and pre-treated with vehicle or labeled inhibitor (10 mg/kg). n = 8 biologically independent mice.

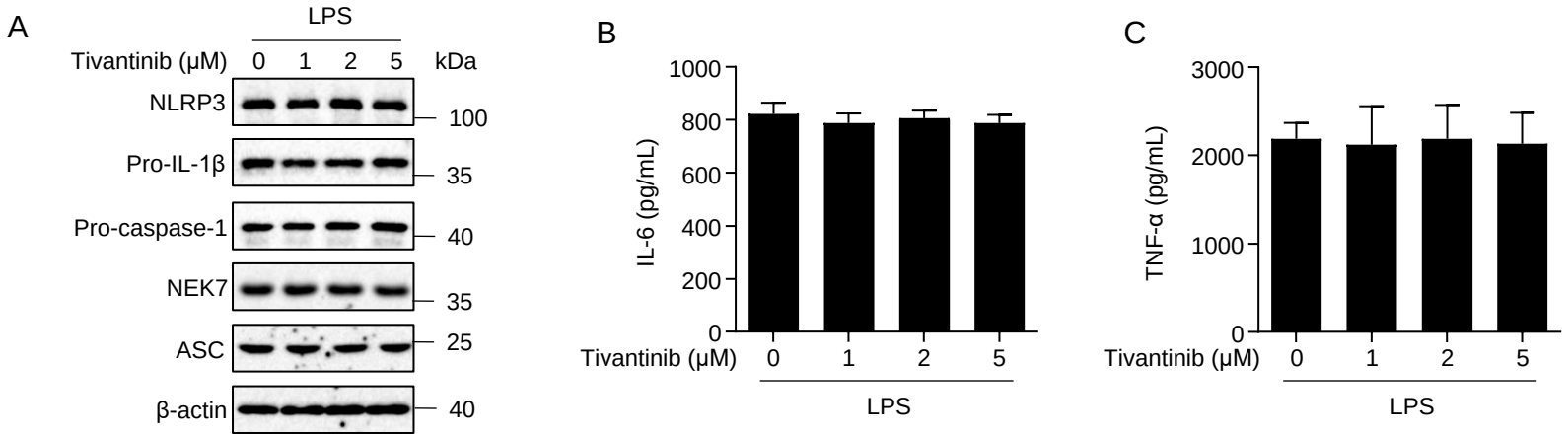

Figure S3. Tivantinib has no effect on the priming step of NLRP3 inflammasome activation, Related to Figure 4. (A) Western blot analysis of NLRP3, NEK7 and ASC in Input of LPS-primed BMDMs treated with various concentrations of Tivantinib (as labeled). (B, C) ELISA analysis of IL-6 (B) and TNF-α (C) in SN of LPS-primed BMDMs treated with various concentrations of Tivantinib (as labeled). Data represent means ± SEM from four biological duplicates (B, C).

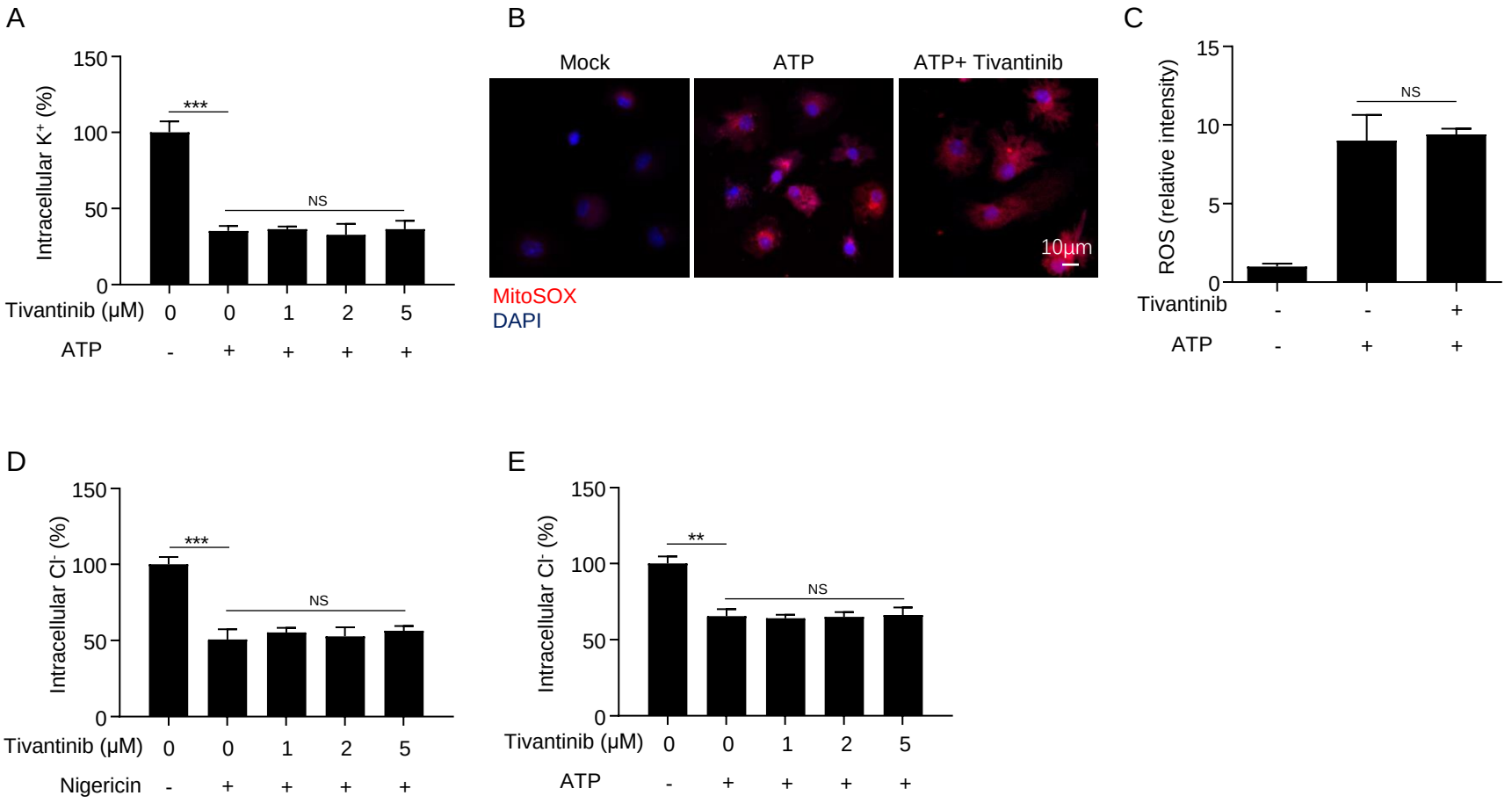

Figure S4. Tivantinib does not block potassium and chloride efflux, and mitochondrial oxidation, Related to Figure 4. (A) Qualification of intracellular potassium level by ICP-OES in LPS-primed BMDMs treated with various concentrations of Tivantinib (as labeled) and then stimulated with 2.5 mM ATP for 30 min. (B) Confocal microscopy analysis in LPS-primed BMDMs treated with 5 μM Tivantinib and then stimulated with 2.5 mM A TP , followed by staining with Mitosox and DAPI. (C) The percentage of relative fluorescence intensity of mitoSOX in LPS-primed BMDMs treated with 5 μM Tivantinib and then stimulated with 2.5 mM ATP. (D, E) Qualification of intracellular chloride level in LPS-primed BMDMs treated with various concentrations of Tivantinib (as labeled) and then stimulated with 5 μM nigericin (D) and 2.5 mM ATP (E) for 15 min. Data represent means ± SEM from four biological duplicates (A, C-E). Statistical analysis was performed using one-way ANOV A (A, D, E) or unpaired Student's t-test (**C**). $^{**}P < 0.01$, $^{***}P < 0.001$.

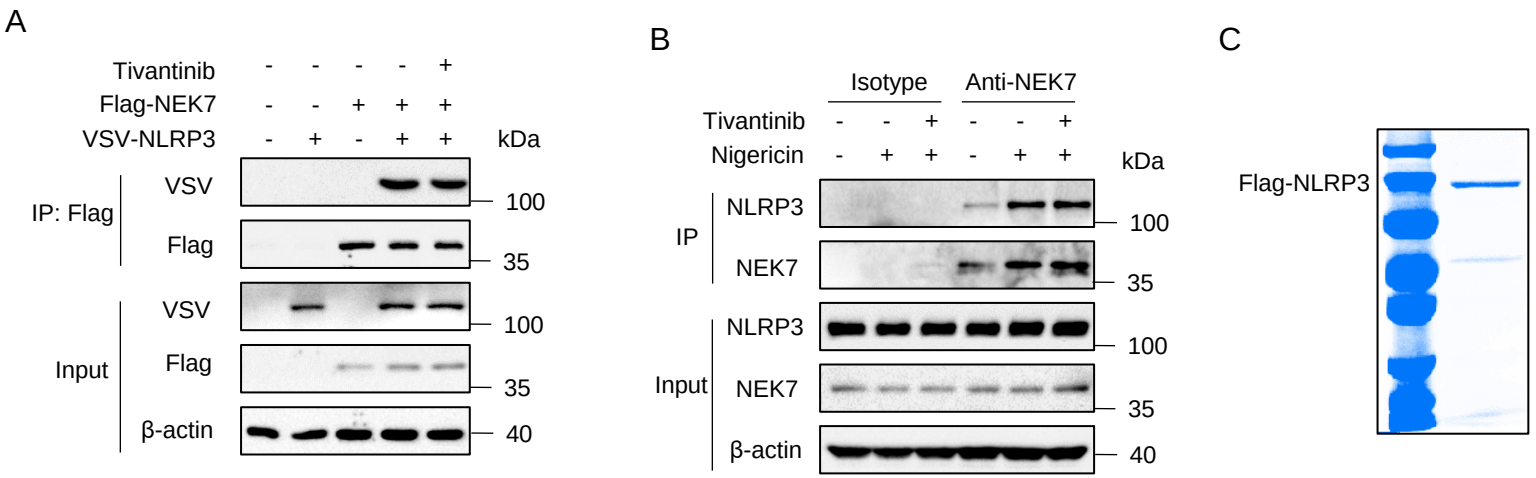

Figure S5. Tivantinib does not block NLRP3-NEK7 interactions, Related to Figure 4. (A) IP and western blot analysis of the interaction of endogenous NEK7 and NLRP3 in LPS-primed BMDMs treated with 5 μM Tivantinib and then stimulated with 5 μM nigericin for 30 min. (B) IP and western blot analysis of the interaction of Flag-NEK7 and VSV-NLRP3 in the lysates of HEK-293T cells treated with 10 μM Tivantinib. (C) Commassie blue stainning of purified NLRP3 protein.

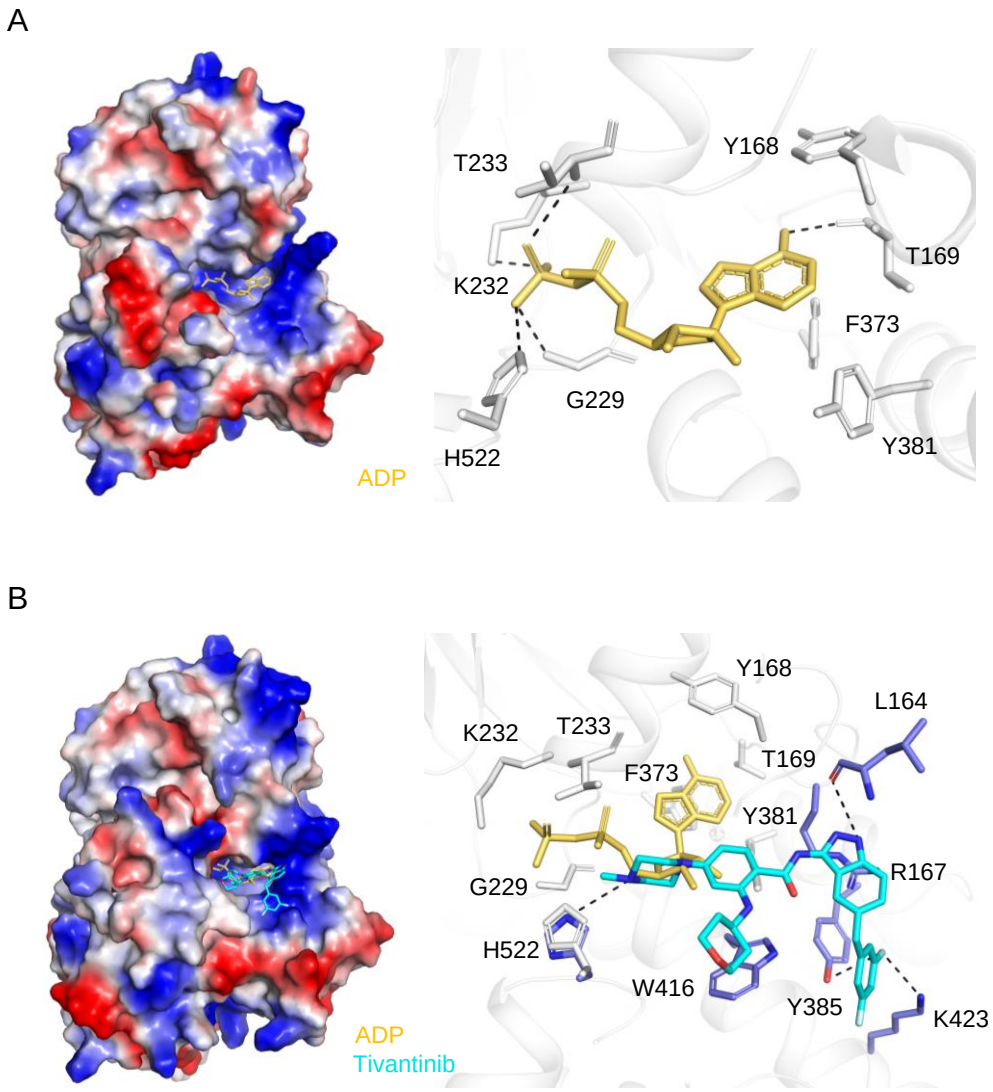

Figure S6. Tivantinib was readily docked into the ATP-binding pocket of NLRP3, Related to Figure 4. (A) Docking complex of NLRP3 with ATP. ATP is shown in sticks and colored yellow, NLRP3 is shown in cartoon and colored light gray, key amino acid residues were shown as sticks. (B) Docking complex of tivantinib with NLRP3 in the ATP-binding pocket. Tivantinib is shown in sticks and colored light green, ATP is shown in sticks and colored yellow, NLRP3 is shown in cartoon and colored light gray, key amino acid residues were shown as sticks.

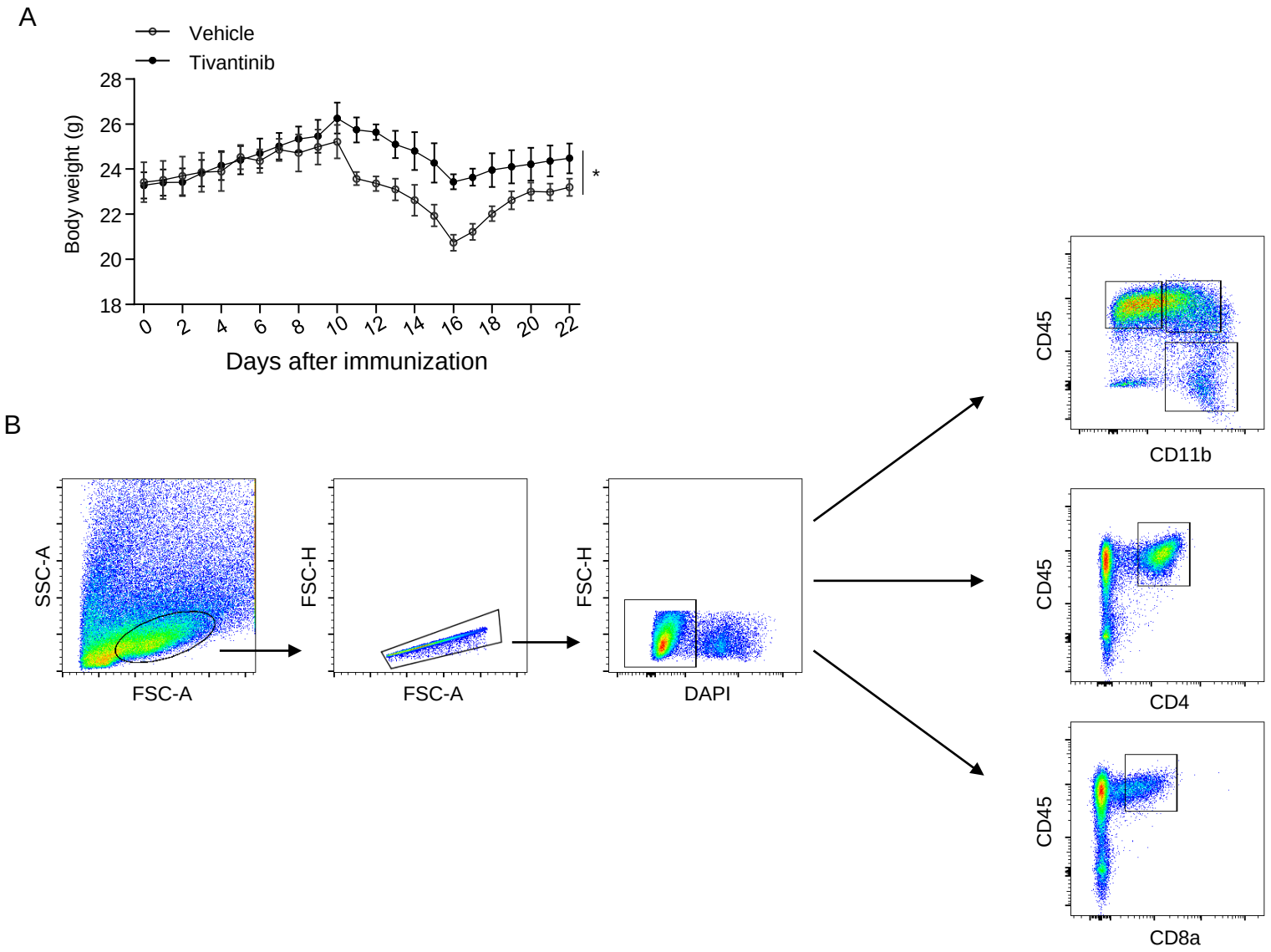

Figure S7. The gating strategies of Flow cytometry, Related to Figure 6. (A) Intraperitoneal injection of vehicle or Tivantinib (10 mg/kg) in C57BL/6J mice beginning at the induction of EAE every 2 days, Loss of weight after EAE induction. (B) The gating strategies of Flow cytometry to analysis the immune cell infiltration in CNS. Data represent means ± SEM. Statistical analysis was performed using one-way ANOVA. *$P < 0.05$.