

## Supplemental material

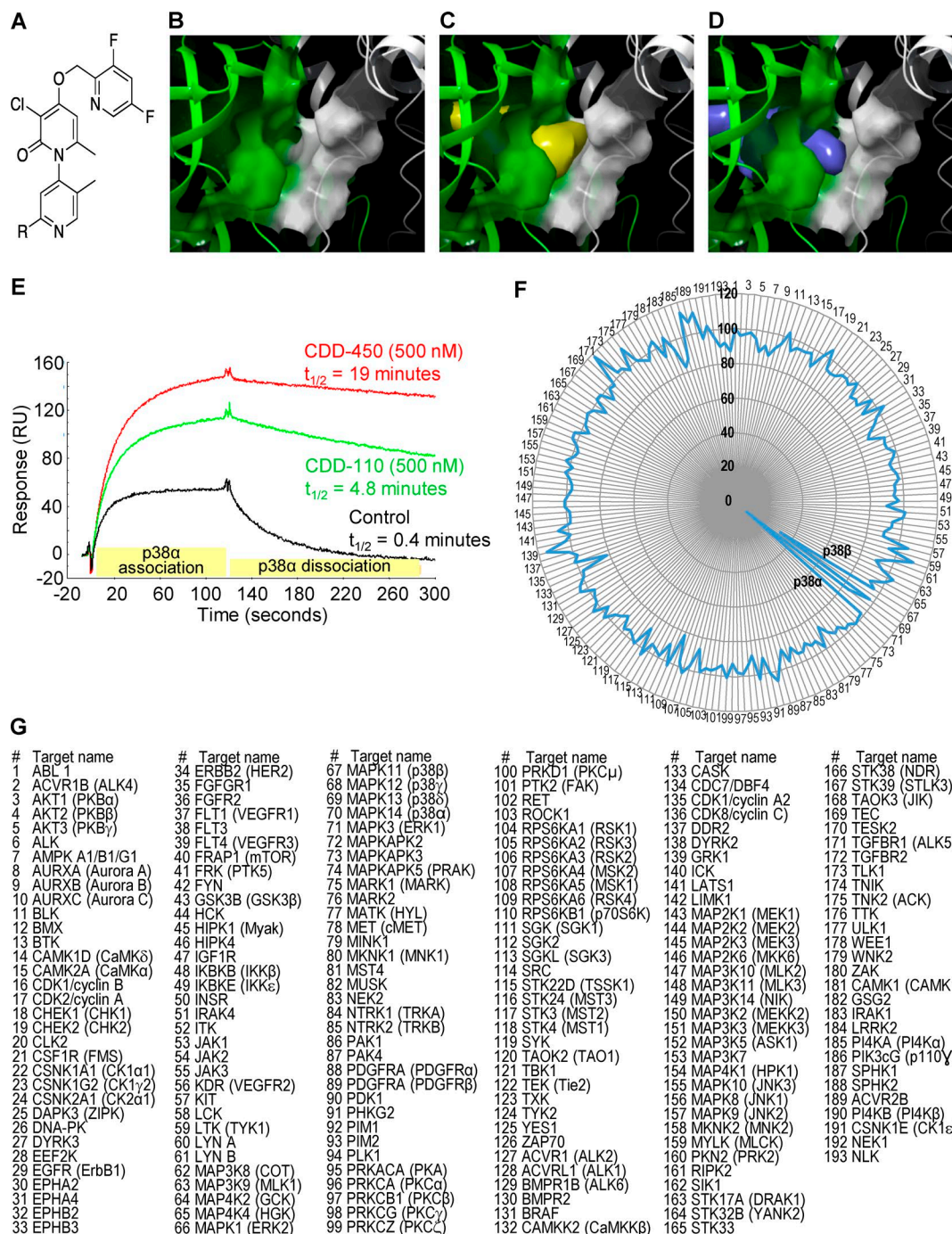
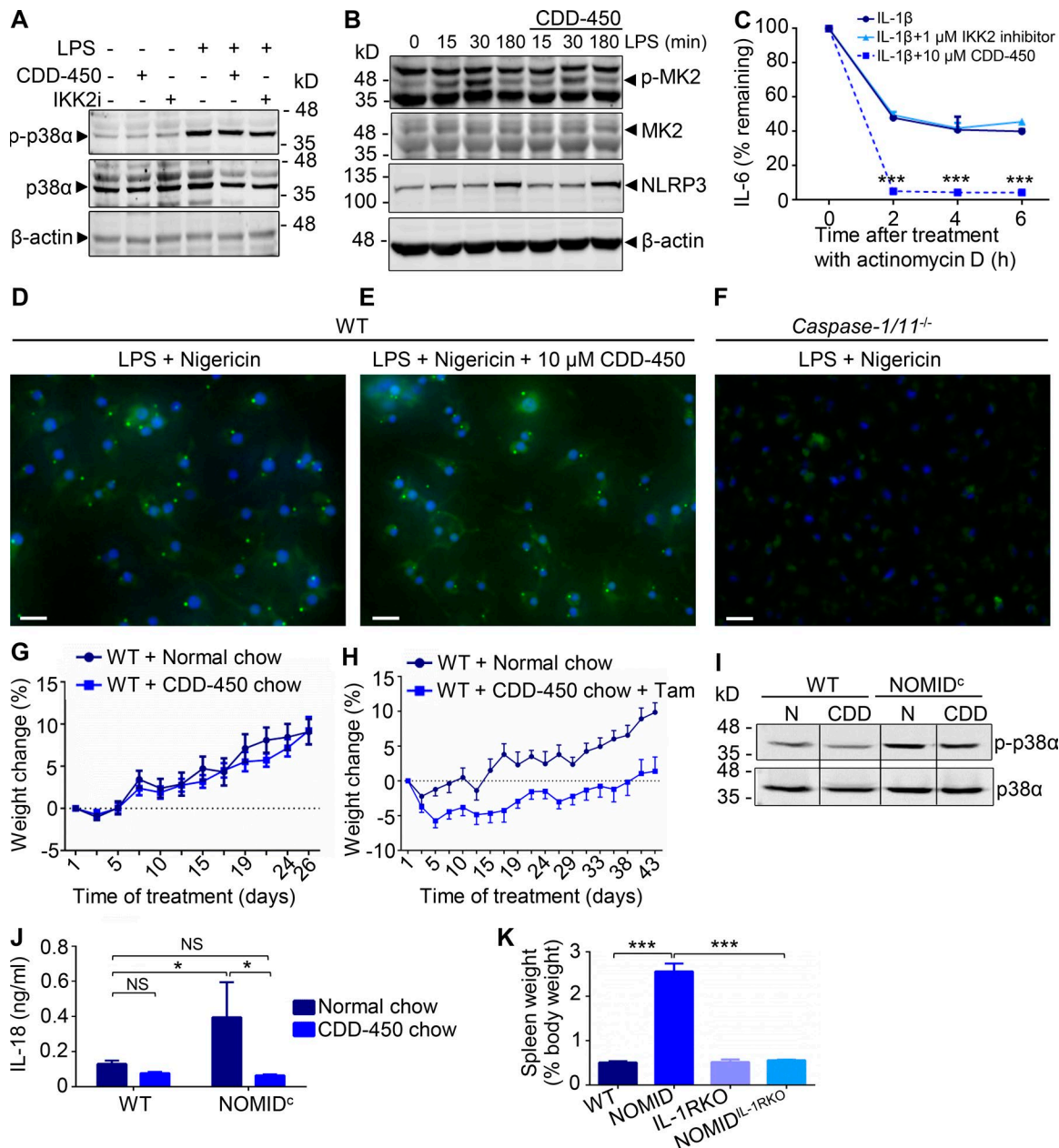
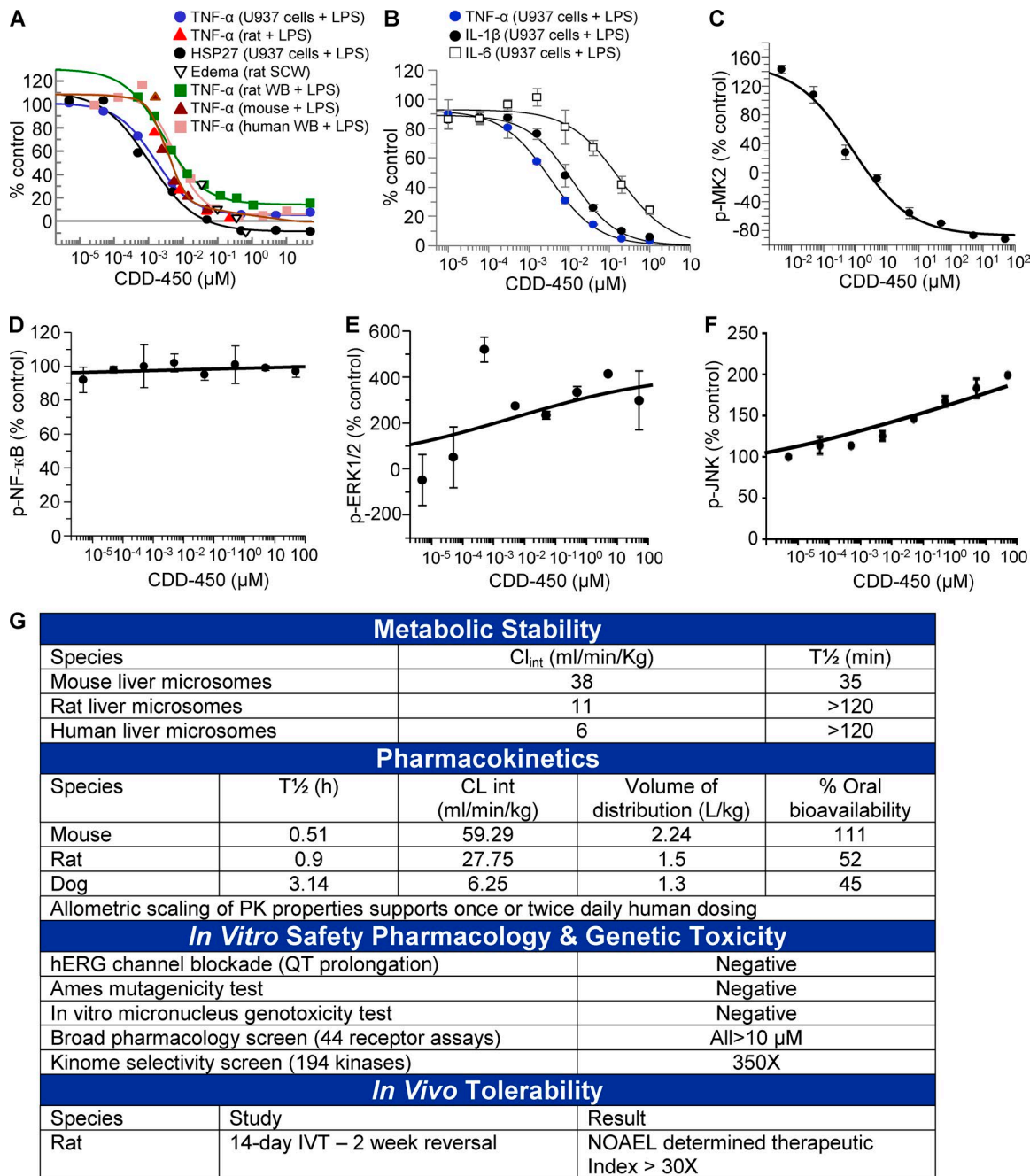
Wang et al., <https://doi.org/10.1084/jem.20172063>

Figure S1. **Binding and selectivity proprieties of CDD-450.** (A) Structure of CDD-450. (B) A ribbon diagram of the p38α (green) and MK2 (white) complex. (C) A view of CDD-450 (yellow) bound to p38α-MK2 complex. (D) A view of CDD-110 (blue) bound to p38α-MK2 complex. (E) Surface plasmon resonance analysis of p38α binding to immobilized MK2 in the absence or presence of CDD-450 or CDD-110. RU, resonance unit. Data are representative of at least two independent experiments. (F) Spider plot representation of CDD-450 selectivity. CDD-450 was tested at 5 μM. (G) Corresponding kinases represented in the Spider plot.



**Figure S2. Effects of inhibitors and deletion of IL-1 receptor on p38α-MK2 signaling, IL-1β and TNF-α mRNA stability, and inflammation. (A and B)** Western blot analysis of the effects of CDD-450 and IKK2 inhibitor (IKK2i; PHA-408) on LPS-induced p38α and MK2 activation in WT mouse BMMs. Cells were preincubated with 10 μM CDD-450 or 1 μM IKK2 for 1 h before stimulation with 100 ng/ml LPS for the indicated times. Data are representative of at least two independent experiments. **(C)** qPCR analysis of CDD-450 effects on IL-6 mRNA stability. WT BMMs were stimulated with 100 ng/ml LPS for 3 h and then simultaneously exposed to 1 μg/ml actinomycin D and CDD-450 or IKK2 inhibitor for 0, 2, 4, or 6 h. **(D–F)** Microscopic analysis of active caspase-1. WT BMMs (D and E) or *caspase-1/11* null BMMs (F) were primed with 100 ng/ml LPS for 3 h and treated with 15 μM nigericin (D and F) or simultaneously with 15 μM nigericin and 10 μM CDD-450 (E) for 30 min and then incubated with the FLICA FAM-YVAD-FMK probe. Treatment with LPS alone showed no fluorescent foci (not depicted). Bars, 10 μm. **(G)** CDD-450 effects on body weight in WT mice. 3-mo-old mice were fed with normal or CDD-450 chow for the indicated times (5–10 mice/group). **(H)** Effects of concomitant administration of CDD-450 and tamoxifen (tam) on body weight. 3-mo-old WT mice were fed with normal or CDD-450 chow (six mice/group) starting 3 d before sustained tamoxifen administration for 2 wk. The experiments were terminated 4 wk later. **(I)** Western blot analysis of total and phosphorylated p38α (p-p38α) in liver extracts. Results are from the same gels, but the lanes were cut and pasted. N, normal chow; CDD, CDD-450 chow. **(J)** Analysis of IL-18 levels in bone marrow. 3-mo-old WT and *NOMID<sup>c</sup>* mice were fed with normal or CDD-450 chow starting 3 d before sustained tamoxifen administration for 2 wk. The experiments were terminated 4 wk later. Bone marrow was centrifuged, and IL-18 levels in the supernatants were measured by ELISA. **(K)** Effect of IL-1 receptor ablation on splenomegaly. 2-wk-old mice (three mice/group) were used. IL-1RKO, IL-1 receptor knockout. In vitro data are representative of at least two independent experiments. Data are means ± SEM from experimental triplicates. \*,  $P < 0.05$ ; \*\*\*,  $P < 0.001$ .



**Figure S3. Properties of CDD-450 and its effects on IL-1β, IL-6, and TNF-α production.** **(A)** Effects of CDD-450 on TNF-α production in in vitro systems (U937 cells, rat whole blood, and human whole blood [WB] stimulated with LPS) and in vivo models (mice or rats treated with LPS and rat SCW). **(B)** Effects of CDD-450 on IL-1β, IL-6, and TNF-α production in U937 cells stimulated with LPS. **(C–F)** Effects of CDD-450 on LPS-stimulated phosphorylation of MK2, NF-κB/p65 (IKK2 pathway), ERK1/2, and JNK in U937 cells. Cytokines, total, and phosphorylated proteins were measured by ELISA or MesoScale Diagnostics, and edema was assessed by a plethysmometer. Data are representative of at least three independent experiments. Data are means from experimental triplicates and are representative of at least three independent experiments. **(G)** Proprieties of CDD-450. Metabolic stability: Mouse (male C57BL/6), rat (male Sprague Dawley), or human liver microsomes were incubated with CDD-450 for various times. The stability of CDD-450 in the samples was determined by liquid chromatography–tandem mass spectrometry. Pharmacokinetics: Various doses of CDD-450 were administered orally, intravenously, and i.p. to mice, rats, and dogs (male beagles). Blood was collected at different times for determinations of CDD-450 concentrations in plasma by liquid chromatography–tandem mass spectrometry and other parameters (CL, clearance intrinsic; volume of distribution and oral bioavailability). In vitro safety pharmacology and genetic toxicity: hERG: the potential of CDD-450 to inhibit hERG channel was evaluated by Thermo Fisher Scientific. Ames mutagenicity test: CDD-450 was evaluated for the potential to induce mutations to several strains of *Salmonella typhimurium* and *E. coli* in the absence or presence of an exogenous metabolic activation system (S9). Micronucleus genotoxicity test: CDD-450 was evaluated for the potential to induce mutations to Chinese hamster ovary-K1 cells in the absence or presence of an exogenous metabolic activation system (S9) by BioReliance. Broad pharmacology and kinome selectivity screens were performed by Confluence Discovery Technologies, Inc. and Thermo Fisher Scientific, respectively. In vivo tolerability: CDD-450 was administered by oral gavage for 14 consecutive days to rats by Seventh Wave Laboratories, Inc. Evaluated endpoints include food consumption, body weight, organ weight, hematology, clinical chemistry, and histopathology. PK, pharmacokinetics; QT, Q and T waves; IVT, in vivo tolerability; NOAEL, no observed adverse effect level.

Table S1. **List of primers for qPCR**

<b>Primers</b>	<b>Sequence (5'-3')</b>
mCyclophilin B forward	AGCATACAGGTCCTGGCATC
mCyclophilin B reverse	TTCACCTTCCCAAGACCAC
mNlrp3 forward	CCACATCTGATTGTGTTAATGGCT
mNlrp3 reverse	GGGCTTAGGTCCACACAGAA
mIl-18 forward	ACAGGCCTGACATCTTCTGC
mIl-18 reverse	ATTGTTCTGGCCAAGAGG
mIl-1 $\beta$ forward	GTGCAAGTGTCTGAAGCAGC
mIl-1 $\beta$ reverse	CAAAGGTTTGAAGCAGCCC
mIl-6 forward	TTCTCTGGGAAATCGTGGAAA
mIl-6 reverse	TGCAAGTGCATCATCGTTGTT
mNek7 forward	GCTGTCTGTATATGAGATGGC
mNek7 reverse	CCGAATAGTGATCTGACGGGAG
mAsc forward	AACTGCGAGAAGGCTATGGG
mAsc reverse	TGAGCTCCAAGCCATACGAC
mCaspase1 forward	GGACCCTCAAGTTTGGCCCT
mCaspase1 reverse	AGACGTGTACGAGTGTTGT
hNlrp3 forward	CGTGAGTCCCATTAAGATGGAGT
hNlrp3 reverse	CCCACAGTGGATATAGAACAGA
hIl-1 $\beta$ forward	CTGAGCTCGCCAGTGAAATG
hIl-1 $\beta$ reverse	TGTCCATGGCCACAACAAC
hIl-18 forward	AAGATGGCTGCTGAACCAGTA
hIl-18 reverse	GGTCCGGGTGCATTATCTC
hIl-6 forward	ACTCACCTCTTCAGAACGAATTG
hIl-6 reverse	CCATCTTTGGAAGGTTTCAGGTTG
hPpia forward	TCCTGGCATCTTGTCATG
hPpia reverse	CCATCCAACCACTCAGTCTTG