Supplemental Material

AKT regulates NLRP3 inflammasome activation by phosphorylating NLRP3 serine 5

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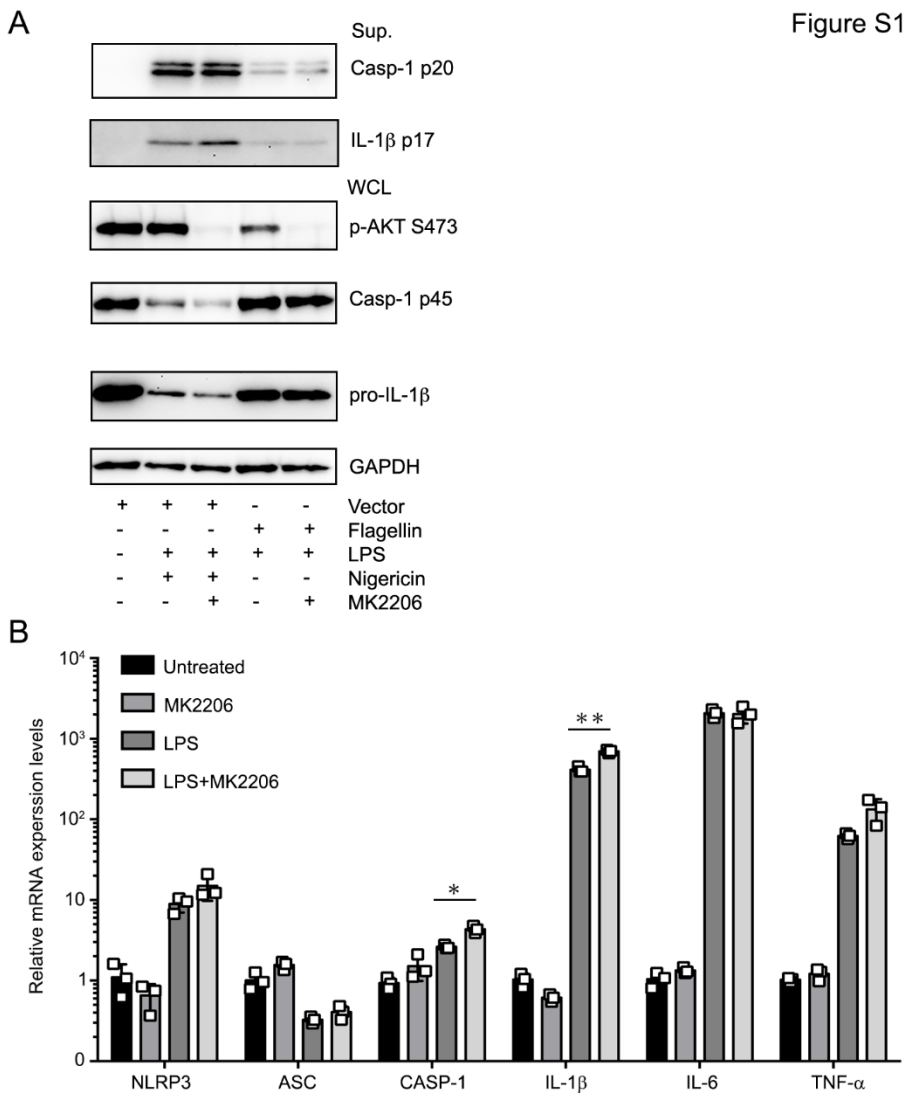

Figure S1. Related to Fig 1. **(A)** Immunoblots of the culture supernatants (Sup) and whole cell lysates (WCL) of PMA differentiated THP-1 cells primed with LPS (50ng/ml, 6h) after activation of the NLRC4 (flagellin) or NLRP3 (nigericin) inflammasomes. **(B)** Activation of p-AKT directly regulates the inflammasome activation. Qualification of the mRNA levels of the indicated genes in THP-1 cells after the indicated stimuli. Experiments were repeated at least three times, and bars represent the means ± SEM. *p<0.05, **p<0.01, as determined by student's t test.

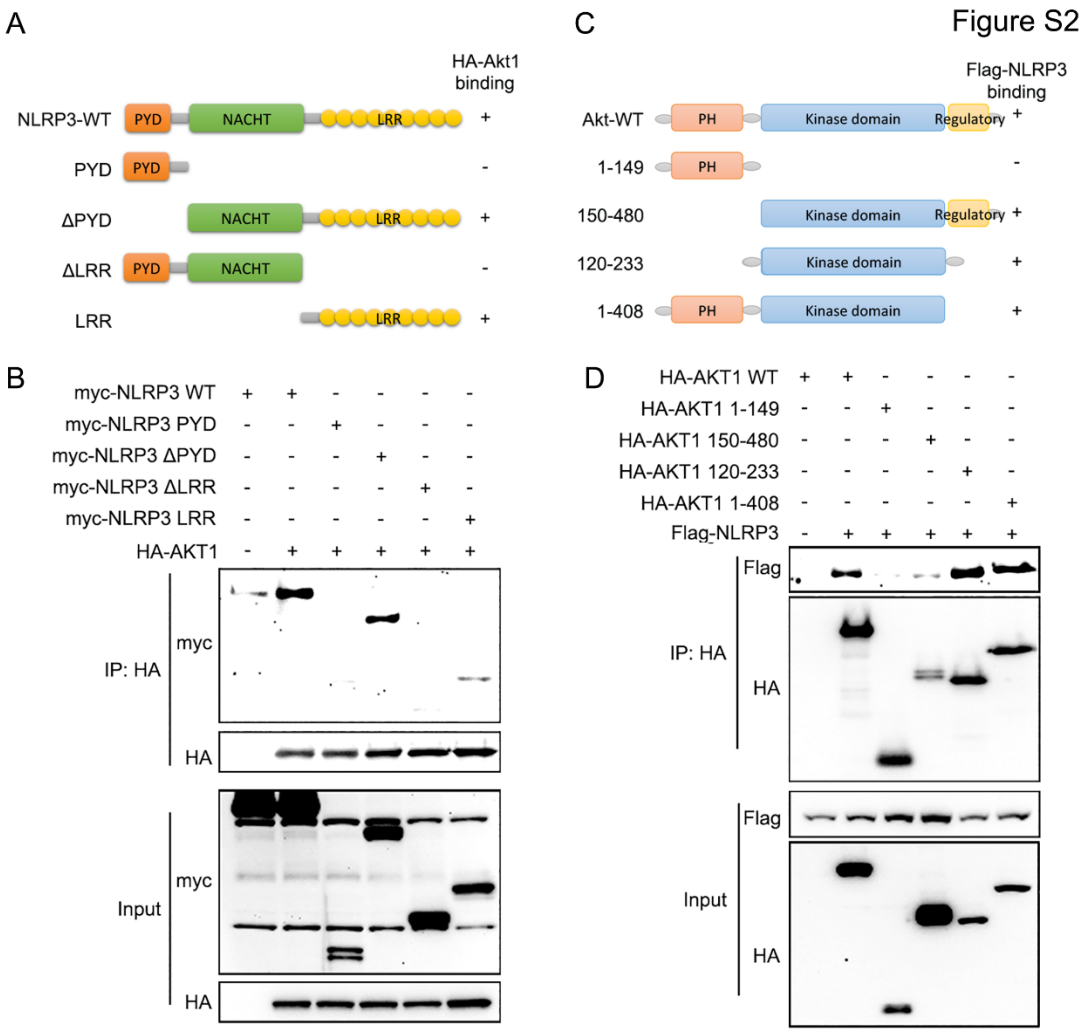

Figure S2. Mapping the interaction between NLRP3 and AKT. **(A)** Schematic diagram of NLRP3 and its truncation mutants. PYD, Pyrin domain; LRR, leucine-rich repeat. **(B)** Immunoprecipitation assay showing that the LRR domain of NLRP3 is required to bind AKT. HA-AKT1 was transfected with myc-tagged NLRP3 WT or its truncated mutants in HEK293T cells. The cell lysates were immunoprecipitated with anti-HA antibodies, and then immunoblotted with the indicated antibodies. **(C)** Schematic diagram of AKT1 and its truncation mutants. PH, pleckstrin homology domain. **(D)** Immunoprecipitation assay showing that the kinase domain of AKT mainly mediates the interaction with NLRP3. Flag-NLRP3 was transfected with HA-tagged AKT1 WT or its truncated mutants in HEK293T cells. The cell lysates were immunoprecipitated with anti-HA antibodies, and then immunoblotted with the indicated antibodies.