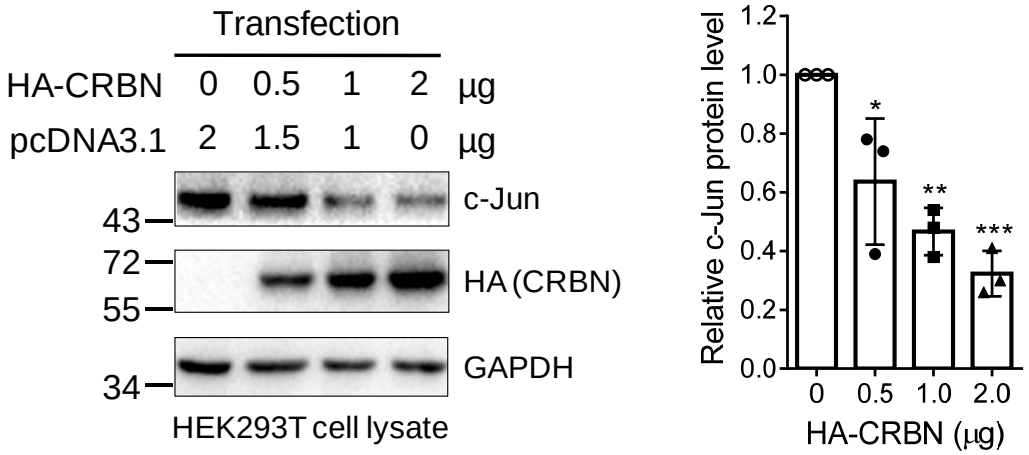
Supplementary Figures

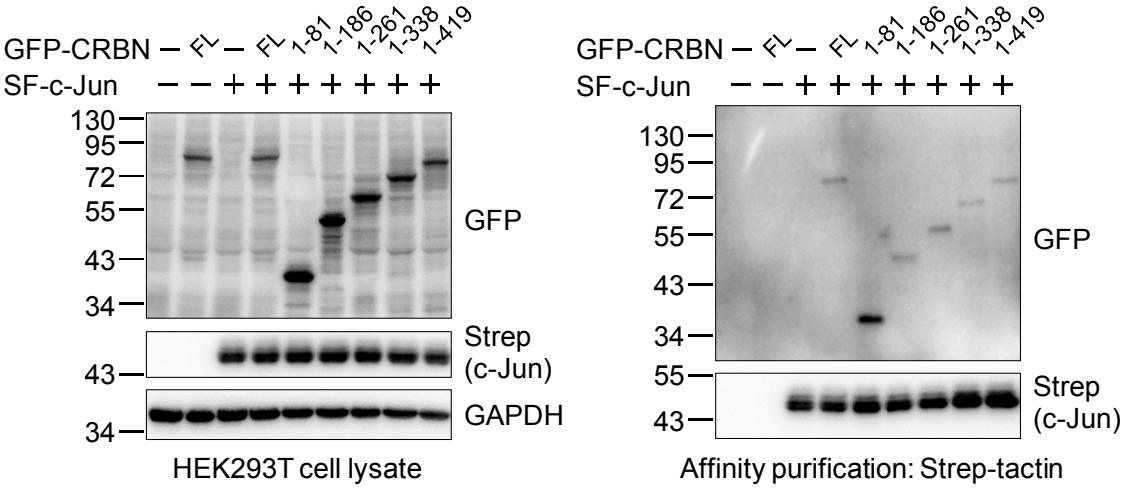
For

Cereblon suppresses lipopolysaccharide-induced inflammatory response through promoting the ubiquitination and degradation of c-Jun

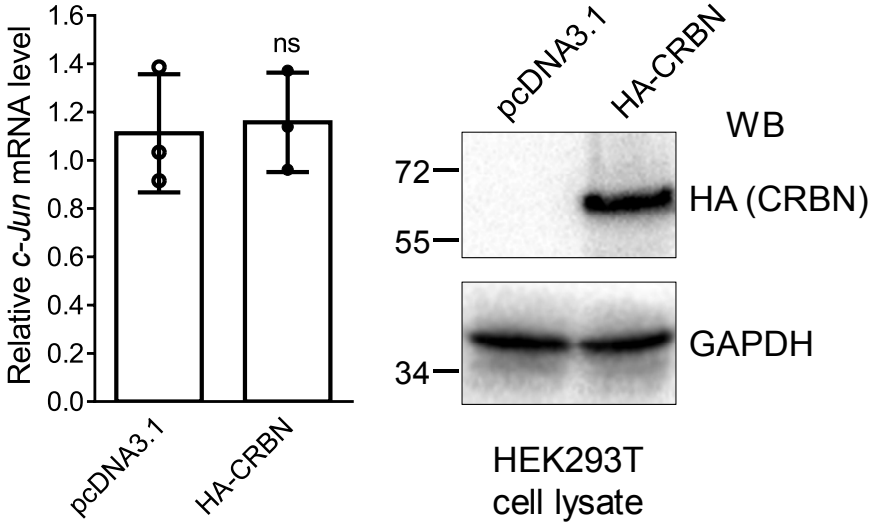
Jing Yang[#], Min Huang[#], Liang Zhou, Xian He, Xiaogang Jiang, Yang Zhang, Guoqiang Xu*



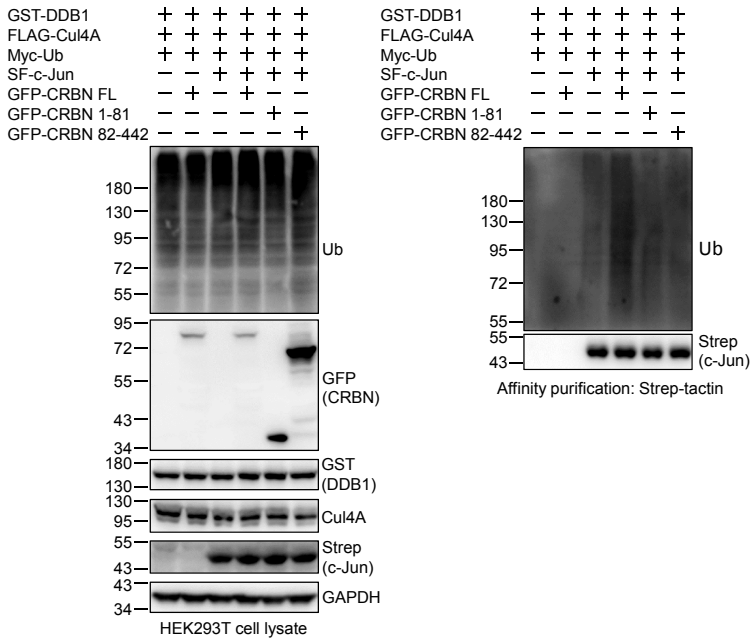
Supplementary Figure S1. CRBN expression decreases c-Jun protein level in a dose dependent manner. HEK293T cells were transfected with the indicated amount of pcDNA3.1 or/and HA-CRBN plasmids in 6-well plates using polyethylenimine (PEI) transfection reagent. Cells were lysed 48 h after transfection and blotted with the indicated antibodies. Three replicates were performed to obtain the mean and standard deviation (SD). Student's *t* test was used to calculate the *P*-value against the control sample. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.



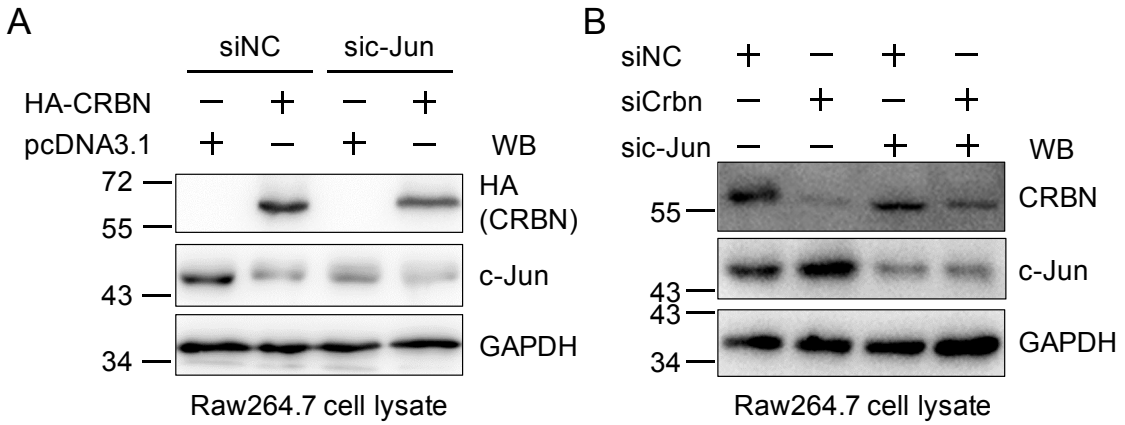
Supplementary Figure S2. CRBN 1-81 domain interacts with c-Jun. HEK293T cells were transfected with pcDNA3.1 or Strep-FLAG (SF)-c-Jun and GFP-CRBN full length (FL), 1-81, 1-186, 1-261, 1-338, or 1-419 domain for 48 h. Then cells were treated with MG132 (10 μM) for 12 h to prevent protein degradation. Cell lysates were prepared and c-Jun was purified with Strep-tactin agarose beads. Immunoblotting was carried out for the cell lysates and affinity purified samples with the indicated antibodies.



Supplementary Figure S3. Expression of CRBN does not affect the *c-Jun* mRNA level. HEK293T cells were transfected with pcDNA3.1 or HA-CRBN plasmid and total RNA was extracted with TRIzol reagents 48 h after transfection. qPCR was performed to measure the relative *c-Jun* mRNA level and *GAPDH* was used as a control for normalization. Three replicates were performed to obtain the mean and SD. Student's *t* test was used to calculate the *P*-value. ns: not significant. Western blotting images showed the CRBN expression. GAPDH was used as a control.



Supplementary Figure S4. CRBN 82-442 domain does not affect the c-Jun ubiquitination. HEK293T cells were transfected with DDB1, Cul4A, ubiquitin, SF-c-Jun, and CRBN or its truncation mutants. pcDNA3.1 was used to balance the total amount of plasmids. At 48 h post-transfection, cells were treated with MG132 (10 μM) for 12 h. SF-c-Jun was purified with Strep-tactin agarose beads. The cell lysate and purified samples were immunoblotted with the indicated antibodies.



Supplementary Figure S5. Western blotting analyses of protein expression for samples used in Figure 5. (A) Raw264.7 cells were transfected with pcDNA3.1 or HA-CRBN and siNC or sic-Jun using lipofectamine 3000 and lysed 48 h after transfection. Cell lysates were immunoblotted with the indicated antibodies (related to Figure 5A-D). (B) Raw264.7 cells were transfected with siNC, siCRBN, and/or sic-Jun using lipofectamine 3000 and lysed 48 h after transfection. Cell lysates were blotted with the indicated antibodies (related to Figure 5E-H).