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Supplementary Materials for

Regulation of the ubiquitylation and deubiquitylation of CREB-binding protein modulates histone acetylation and lung inflammation

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Published 13 June 2017, *Sci. Signal.* **10**, eaak9660 (2017)
DOI: 10.1126/scisignal.aak9660

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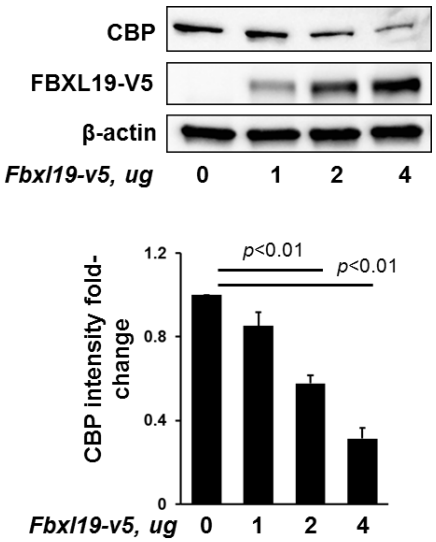

Fig. S1. FBXL19 induces CBP degradation. HBEpCs were transfected with the indicated amounts of *Fbxl19-v5* plasmid. Top: Forty-eight hours later, cell lysates were analyzed by Western blotting with antibodies against CBP, V5, and β-actin. Bottom: Analysis of relative CBP protein abundance was determined by densitometric analysis of band intensities with ImageJ software. Data are means ± SEM of three experiments. *P* values were calculated by one-way ANOVA and post hoc Tukey's test. Western blots are from a single experiment and are representative of at least three independent experiments.

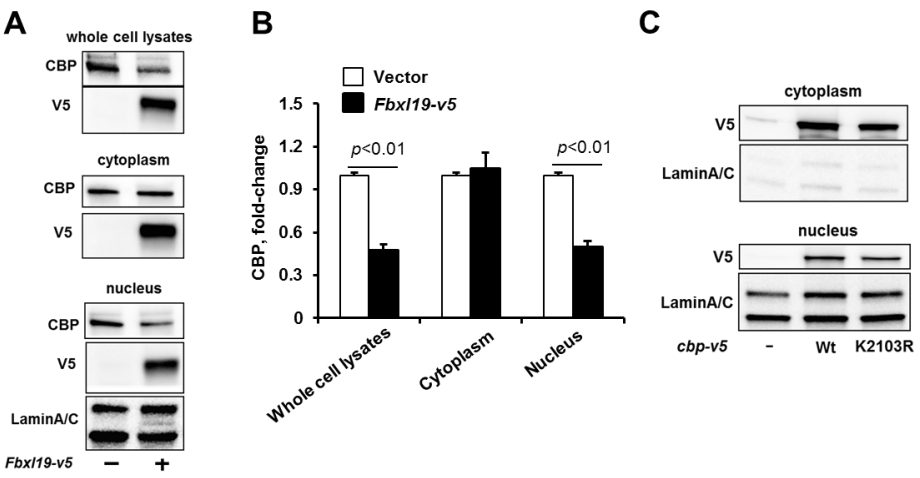

Fig. S2. FBXL19 mediates CBP degradation in the nucleus. (**A** and **B**) MLE12 cells were left untransfected (-) or were transfected with *Fbxl19-v5* plasmid (+). Forty-eight hours later, cell nuclear and cytoplasmic fractions were separated and subjected to Western blotting analysis with antibodies against CBP, V5, and LaminA/C. Whole-cell lysates were also analyzed (A). The relative abundances of CBP protein were determined by densitometric analysis of band intensities with ImageJ software. Data are means ± SEM of three experiments. *P* values were calculated with the unpaired Student's *t* test (B). (**C**) MLE12 cells were left untransfected (-) or were transfected with *cbpwt-v5* or cbp^{K2103R}*-v5* plasmid. Forty-eight hours later, cell nuclear and cytoplasmic fractions were separated and subjected to Western blotting analysis with antibodies against V5 and LaminA/C. Western blots are from a single experiment and are representative of at least three independent experiments.

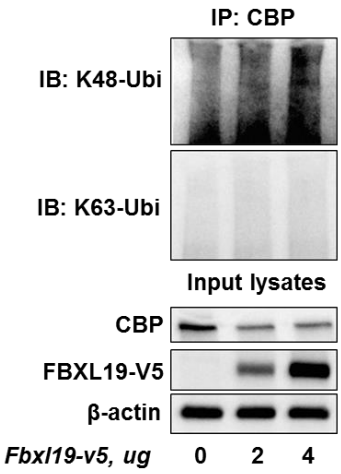

Fig. S3. FBXL19 induces the K48-linked polyubiquitylation of CBP. HBEpCs were transfected with the indicated amounts of *Fbxl19-v5* plasmid. Forty-eight hours later, cell lysates were subjected to immunoprecipitation (IP) with an anti-CBP antibody, which was followed by Western blotting (IB) analysis with antibodies against K48-linked ubiquitin (K48-Ubi) and K63-linked ubiquitin (K63-Ubi). Input cell lysates were analyzed by Western blotting with antibodies against CBP, V5, and β-actin. Western blots are from a single experiment and are representative of at least three independent experiments.

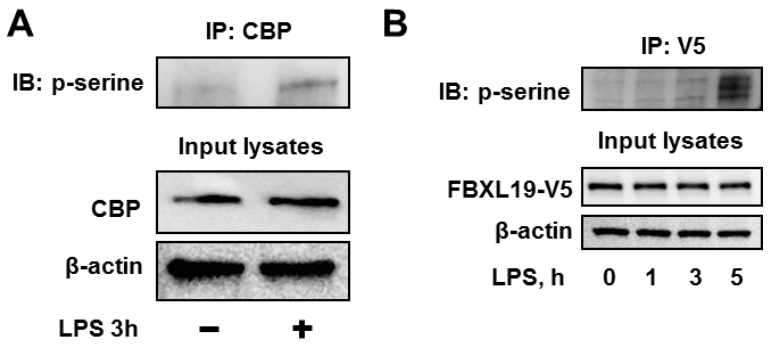

Fig. S4. LPS stimulates the phosphorylation of CBP and FBXL19. (**A**) MLE12 cells were left untreated (-) or were treated with LPS (10 μg/ml) for 3 hours. Cell lysates were then subjected to immunoprecipitation (IP) with an anti-CBP antibody, which was followed by Western blotting (IB) analysis with an antibody against phosphorylated serine residues (p-serine). Input cell lysates were analyzed by Western blotting with antibodies against CBP and β-actin. (**B**) FBXL19-V5-overexpressing MLE12 cells were left untreated or were treated with LPS (10 μg/ml) for the indicated times. Cell lysates were subjected to immunoprecipitation (IP) with an anti-V5 antibody, which was followed by Western blotting (IB) analysis with an antibody against phosphorylated serine residues (p-serine). Input cell lysates were analyzed by Western blotting with antibodies against V5 and β-actin. Western blots are from a single experiment and are representative of at least three independent experiments.

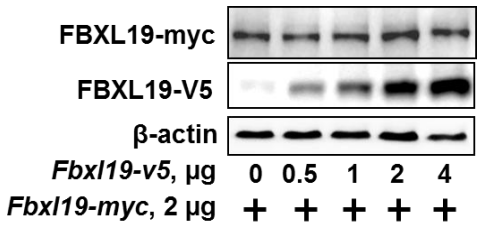

Fig. S5. FBXL19 is not auto-ubiquitylated. MLE12 cells were transfected with 2 µg of *Fbxl19-myc*-plasmid together with the indicated amounts of *Fbxl19-v5* plasmid. Forty-eight hours later, cell lysates were analyzed by Western blotting with antibodies against Myc, V5, and β-actin. Western blots are from a single experiment and are representative of at least three independent experiments.

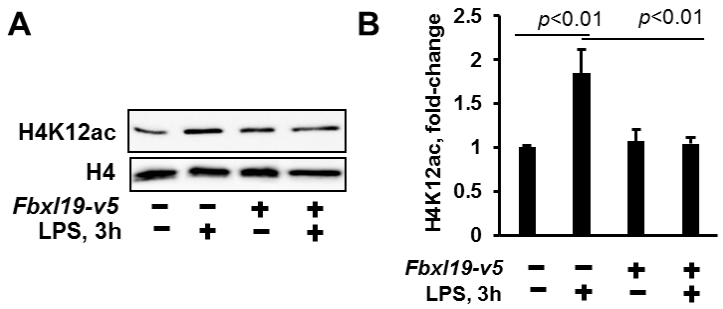

Fig. S6. The reduction in CBP stability attenuates the LPS-induced acetylation of histone H4 on Lys^{12}. (**A** and **B**). MLE12 cells were left untransfected or were transfected with *Fbxl19-v5* plasmid. Forty-eight hours later, the cells were left untreated (-) or were treated with LPS (10 μg/ml) for 3 hours. Histones were extracted and the samples were analyzed by Western blotting with antibodies against histone H4K12ac and histone H4 (A). Fold-changes in the relative abundance of H4K12ac were determined by densitometric analysis of band intensities with ImageJ software (B). Data are means ± SEM of three experiments. *P* values were calculated by two-way ANOVA and post hoc Tukey's test.

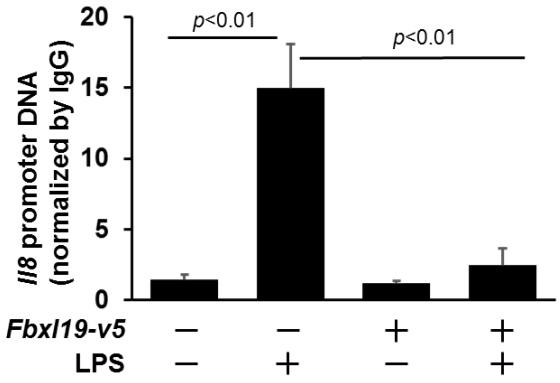

Fig. S7. FBXL19 attenuates histone H4K8ac–mediated *Il8* promoter activity. Beas2B cells were left untransfected (-) or were transfected with *Fbxl19-v5* plasmid. Forty-eight hours later, the cells were left untreated (-) or were treated with LPS (10 µg/ml) for 3 hours. Chromatin immunoprecipitation (ChIP) assays were performed to investigate the extent of binding of H4K8ac to the *Il8* promoter. The relative abundance of DNA from the *Il8* proximal promoter region was determined by real-time PCR analysis as described in Materials and Methods. Data were normalized to the IgG control and are means ± SEM of three experiments. *P* values were determined by two-way ANOVA and post hoc Tukey's test.

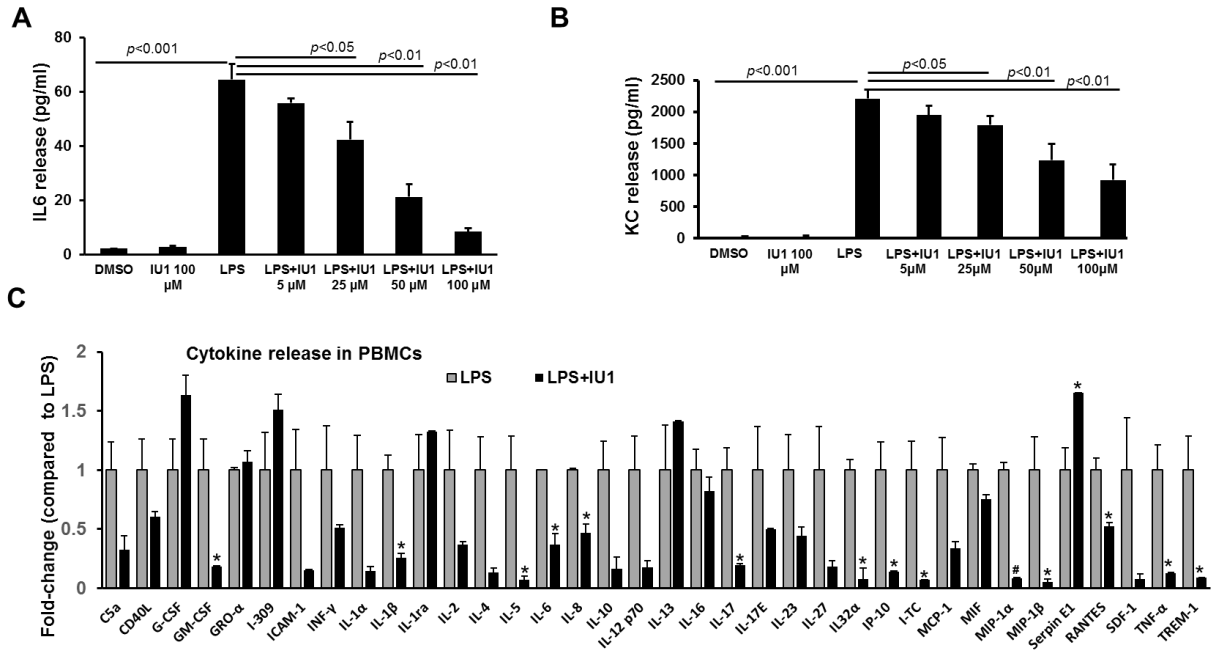

Fig. S8. IU1 attenuates LPS-stimulated cytokine release. (**A** and **B**) MLE12 cells were incubated with IU1 before being treated with LPS (10 µg/ml) for 6 hours. The amounts of IL-6 (A) and KC (B) secreted into the cell culture medium were measured by ELISA. Data are means ± SEM of three experiments. *P* values were calculated by one-way ANOVA and post hoc Tukey's test. (**C**) PBMCs were treated with LPS (0.2 µg/ml) with or without 50 µM IU1 for 24 hours. Cytokine release assays were performed as described in Materials and Methods. Cytokine release by IU1-treated cells was normalized to that released by cells treated with LPS alone. Data are means ± SEM of three experiments. *P* values were calculated by unpaired Student's *t* test. **P* < 0.05 and #*P* < 0.01.