

Expanded View Figures

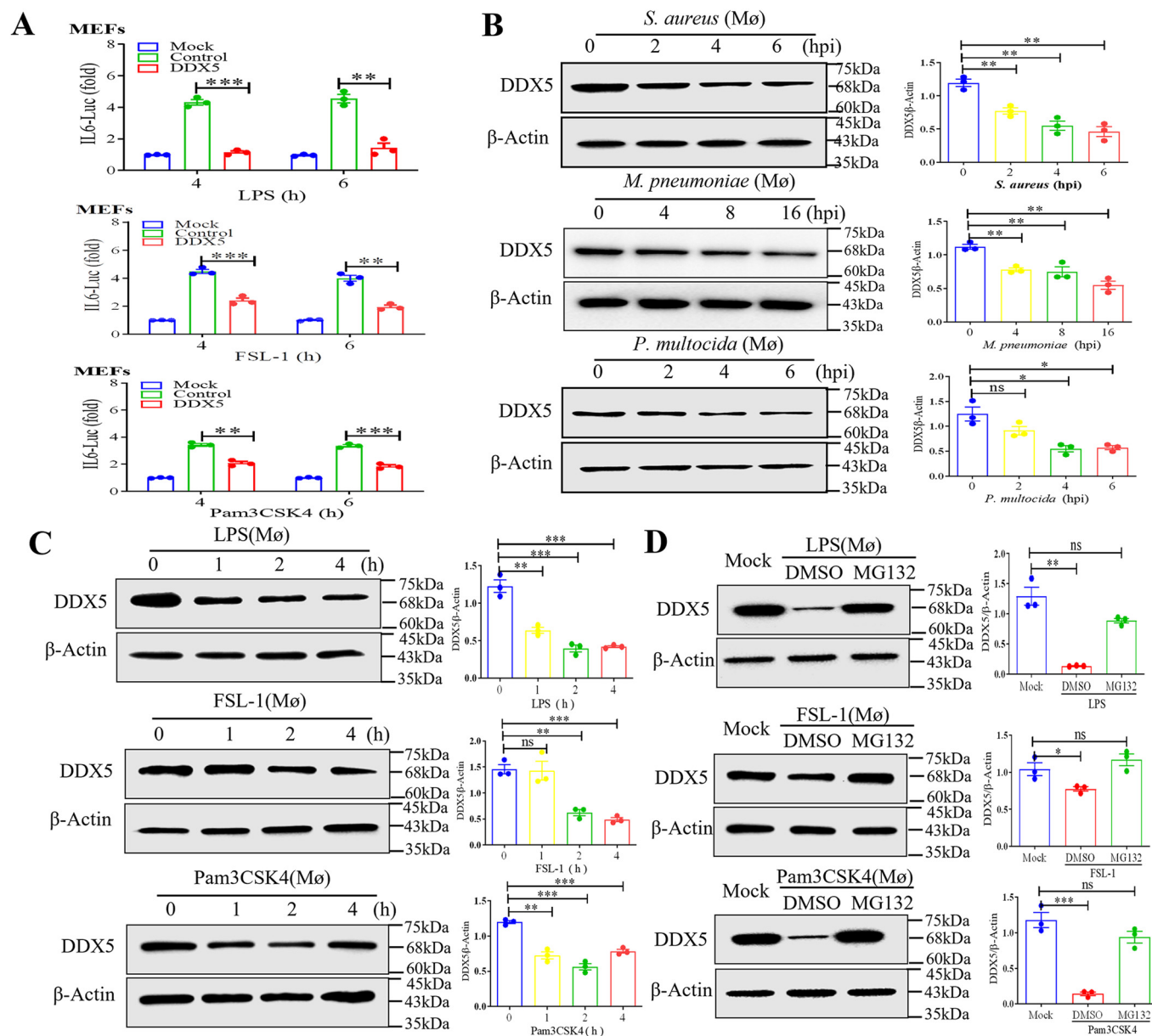
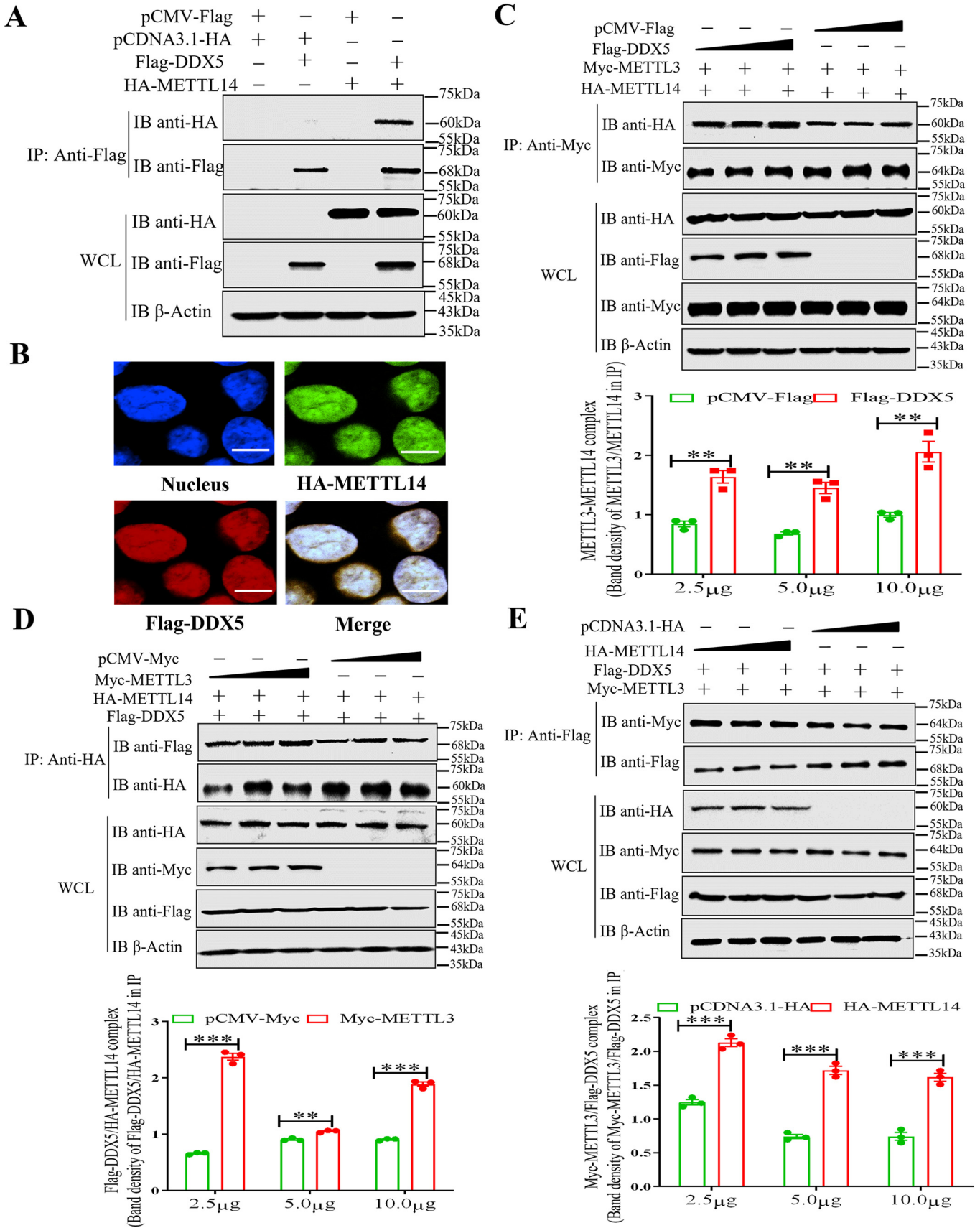


Figure EV1. Luciferase activity of the IL-6 promoter in DDX5-overexpressing MEFs and the degradation of DDX5 via K48 linked ubiquitination in Mø treated with pathogenic bacteria or TLR2/4 agonists.

(A) Luciferase activity of the IL-6 promoter in MEFs treated with LPS, FSL-1, and Pam3CSK4 for 4 and 6 h. (B) DDX5 expression in Mø infected with *S. aureus* for 0, 2, 4, and 6 h; *M. pneumoniae* for 0, 4, 8, and 16 h; *P. multocida* for 0, 2, 4, and 6 h. β-Actin as a reference control. (C) DDX5 expression in Mø treated with LPS, FSL-1, and Pam3CSK4 for 0, 1, 2, and 4 h. (D) DDX5 level in Mø treated with the MG132 proteasome inhibitor, as well as LPS, FSL-1, and Pam3CSK4 for 8 h; DMSO served as the control. The expression of DDX5 was quantified by the band intensity of DDX5/β-Actin in the western blot; the band intensity was measured by Image J software. Data information: In (A–D), all data are represented as the mean ± SEM of three biologically independent samples. "ns" indicates no significant difference, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ (Student's *t* test).



◀ Figure EV2. The interplay and stability of the DDX5-METTL3-METTL14 complex in 293T cells.

(A) Interaction between Flag-DDX5 and HA-METTL14 in 293T cells detected by Co-IP. (B) Co-localization of HA-METTL14 and Flag-DDX5 in 293T cells. Scale bars: 10 μ m. (C-E) The interplay of METTL3, METTL14, and DDX5 in 293T cells. Interaction between Myc-METTL3 and HA-METTL14 in 293T cells transfected with different doses of Flag-DDX5, Myc-METTL3, HA-METTL14 or control vector (2.5, 5.0, and 10.0 μ g). The interaction of Myc-METTL3 and HA-METTL14 (C), HA-METTL14 and Flag-DDX5 (D), or Myc-METTL3 and Flag-DDX5 (E) was quantified by the band intensity of Myc-METTL3/HA-METTL14, Flag-DDX5/HA-METTL14, Myc-METTL3/Flag-DDX5 in the IP system; the band intensity was measured by Image J software. Data information: In (C-E), all data are represented as the mean $\pm$ SEM of three biologically independent samples. ** $p < 0.01$ and *** $p < 0.001$ (Student's t test).



(A, B) TLR4/TLR2 protein levels in NC, siDDX5/siMETTL3 MEFs (A), or in DDX5/METTL3- overexpressing MEFs (B) treated with LPS, FSL-1 and Pam3CSK4 for 4 and 6 h, respectively, and determined by western blot. The expression of TLR4 and TLR2 were quantified by the band intensity of DDX5/ β -Actin in the western blot; the band intensity was measured by Image J software. (C) TLR2/4 localization and expression in GFP control or GFP-DDX5 expressing MEFs. MEFs were transfected with GFP control or GFP-DDX5 expressing plasmid for 24 h, then MEFs were treated with LPS, FSL-1, or Pam3CSK4, respectively; TLR4/TLR2 were detected in MEFs by IFA and CLSM. The green fluorescence indicated GFP (Control)/GFP-DDX5 (DDX5), and the red fluorescence indicated TLR4/TLR2. The right panels show the green line (Control) and red (DDX5) pixel intensity of the white markings in the pictures. Scale bars: 10 μ m. (D, E) The production of IL-6 and TNF- α in primary BMDM following treatment with TLR2/4 agonists. IL-6 (I) or TNF- α (J) production in primary DDX5^{+/+} BMDM or DDX5^{+/-} BMDM treated with LPS, FSL-1, or Pam3CSK4 for 4 and 6 h ($n = 3$). Data information: In (A, B, D, E), all data are represented as the mean $\pm$ SEM of three biologically independent samples. ND not detected. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ (Student's t test).

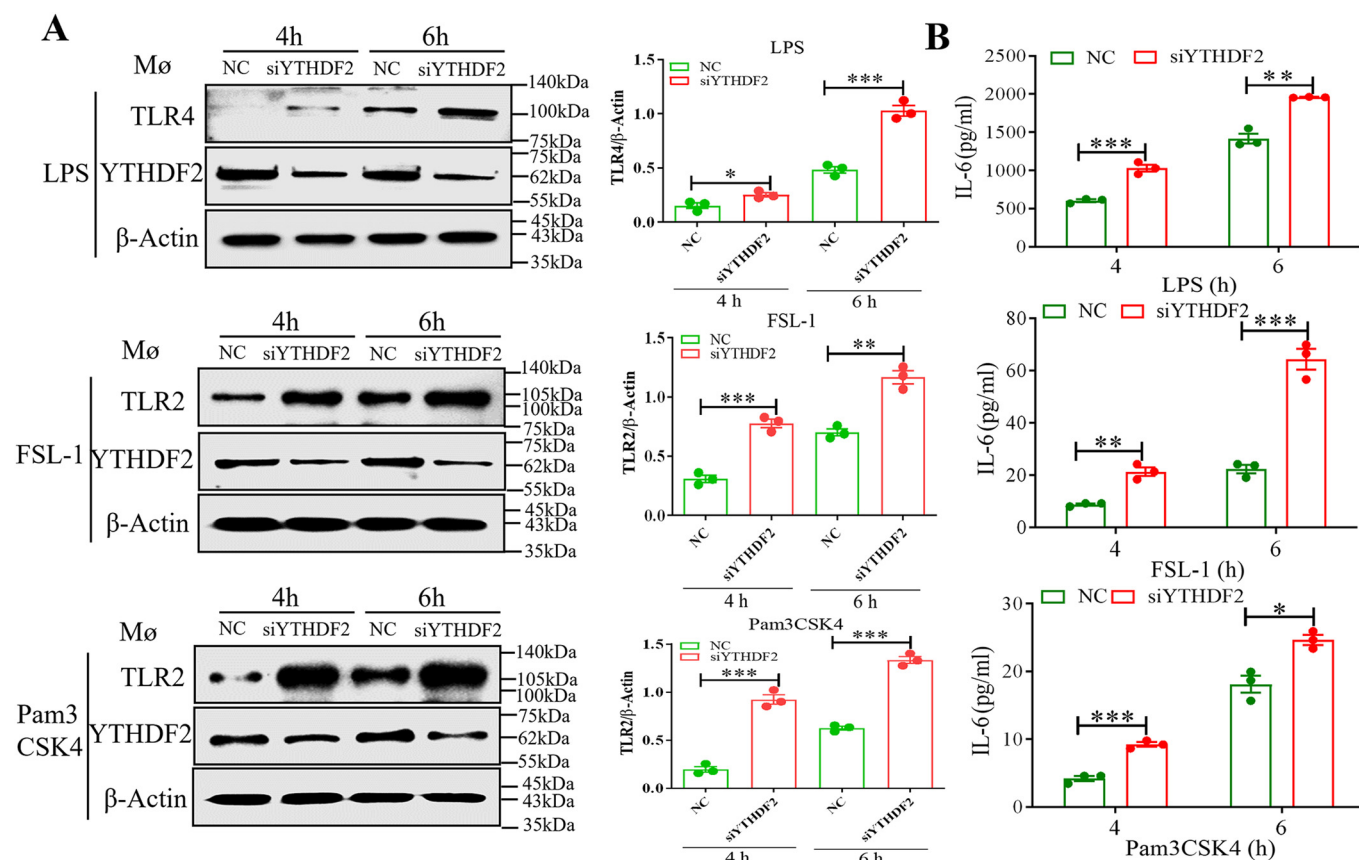


Figure EV4. Expression of TLR2/TLR4 and IL-6 production in YTHDF2 knockdown mouse macrophages (Mφ) treated with LPS, FSL-1, and Pam3CSK4.

(A) TLR4/TLR2 protein level in NC and siYTHDF2 mouse macrophages (Mφ) treated with LPS, FSL-1, or Pam3CSK4 for 4 or 6 h. (B) IL-6 production in NC and siYTHDF2 mouse macrophages (Mφ) treated with LPS, FSL-1, and Pam3CSK4. The expression of TLR4 and TLR2 were quantified by the band intensity of TLR4/β-Actin or TLR2/β-Actin in the western blot; the band intensity was measured by Image J software. Data information: In (A, B), all data are represented as the mean ± SEM of three biologically independent samples. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ (Student's t test).

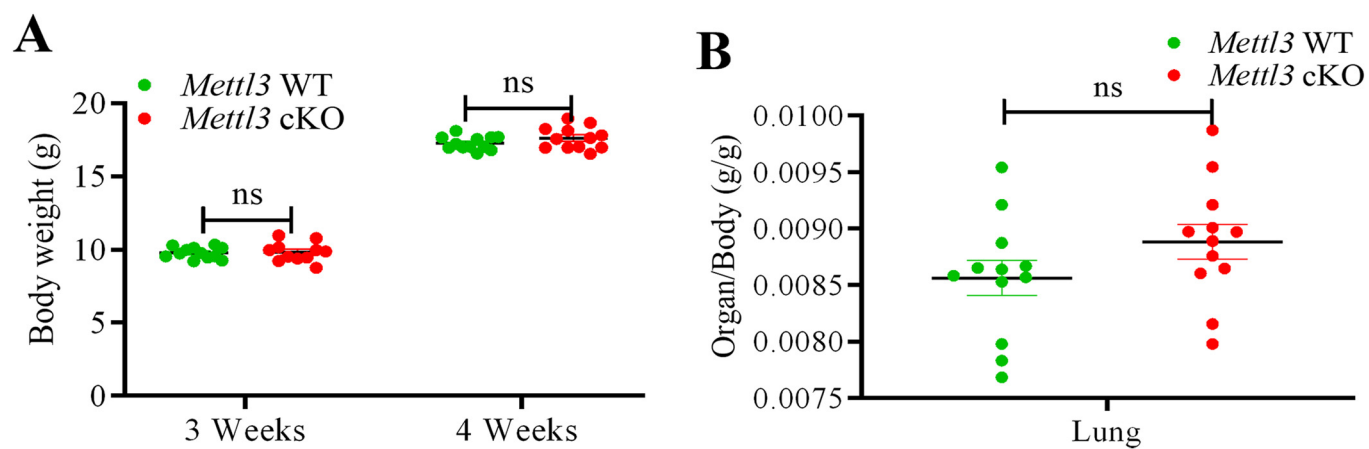


Figure EV5. Quantification of the organ-body rate of *Mettl3* cKO and *Mettl3* WT mice.

(A) Quantification of *Mettl3* WT or *Mettl3* cKO mice at postnatal 3 and 4 weeks ($n = 12$). (B) Organ-body rate was quantified between *Mettl3* WT and *Mettl3* cKO littermates ($n = 12$). Data information: In (A, B), all data are represented as the mean $\pm$ SEM of 12 biologically independent samples. "ns" indicates no significant difference (Student's t test).