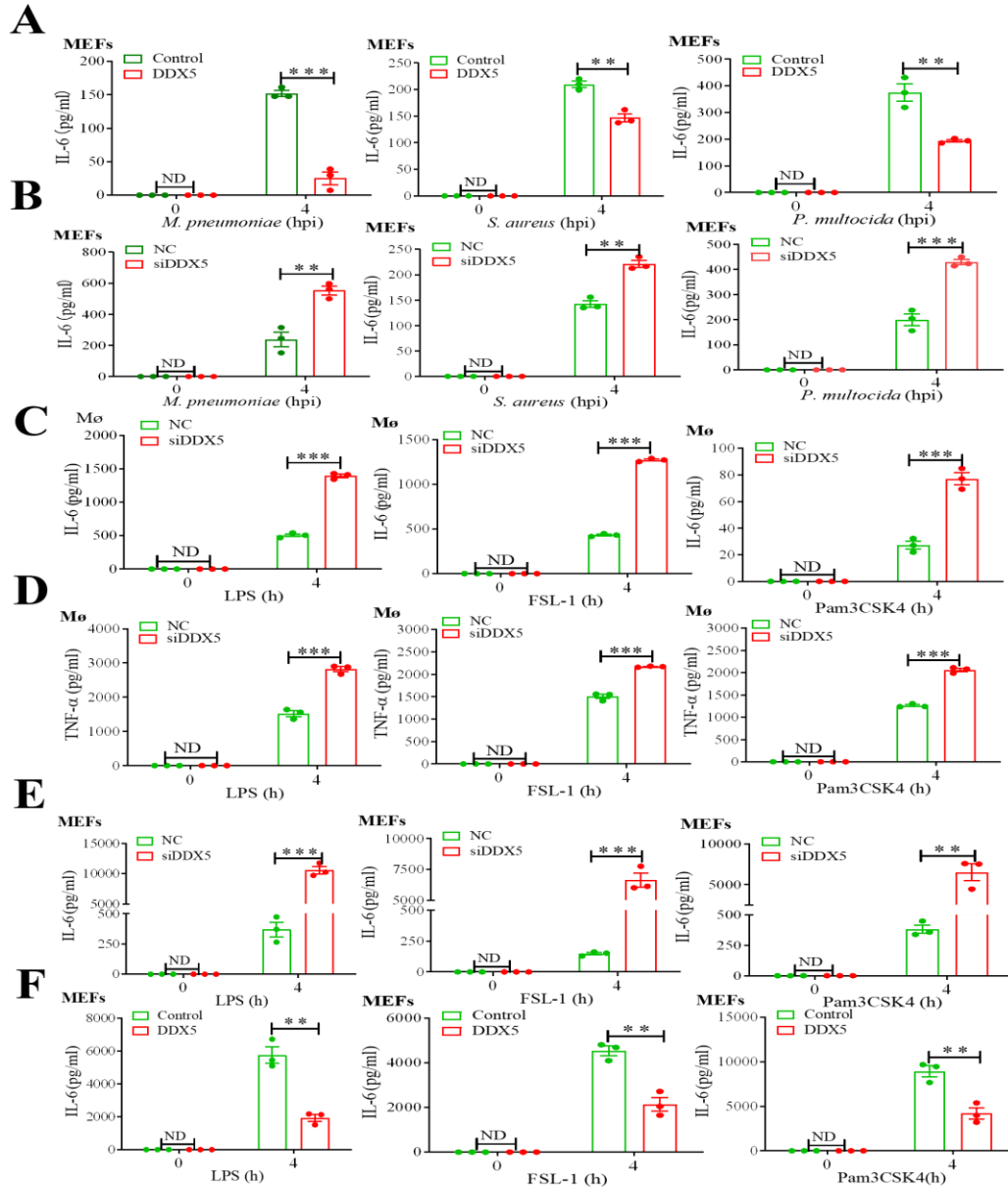


Appendix

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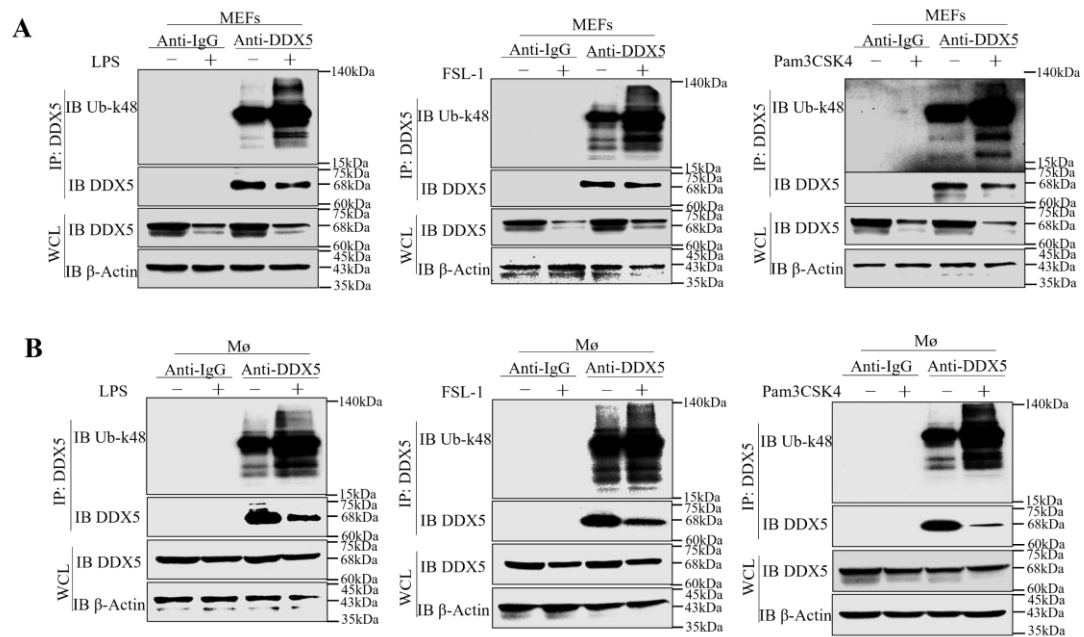
Appendix Figure S1. DDX5 suppresses the production of IL-6 and TNF- α triggered by pathogenic bacteria or TLR2/4 agonists, related to Figure 1.

A-B: IL-6 production in control and DDX5-overexpressing MEFs (A) or in NC and siDDX5 MEFs (B) infected with *P. multocida* or *S. aureus*, *M. pneumoniae* for 4 h (n = 3).

C-D: IL-6 (C) or TNF- α (D) production in NC and siDDX5 Mø treated with LPS, FSL-1, and Pam3CSK4 for 4 h (n = 3).

E-F: IL-6 production in NC and siDDX5 MEFs (E) or in control and DDX5-overexpressing MEFs (F) treated with LPS, FSL-1, and Pam3CSK4 for 4 h (n = 3).

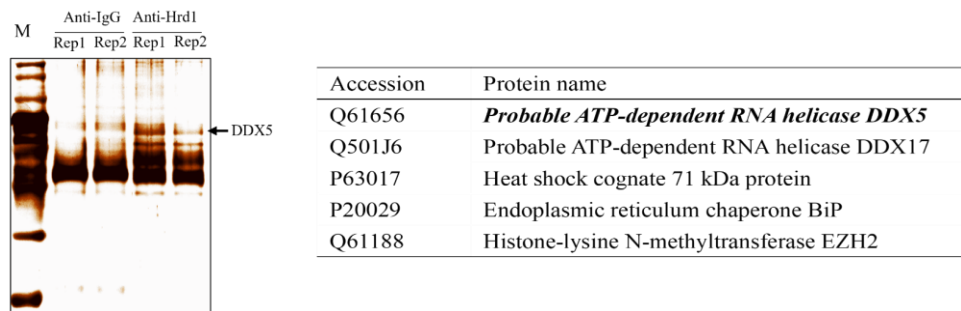
Data information: In (A-F), all data are represented as the mean $\pm$ SEM of three biologically independent samples. ND, not detected. “ns” indicates no significant difference, *p < 0.05, **p < 0.01, and ***p < 0.001 (Student’s t-test).



Appendix Figure S2. Bacterial infection led to DDX5 depression in K48-linked ubiquitin modification, related to Figure 2 and Figure EV1.

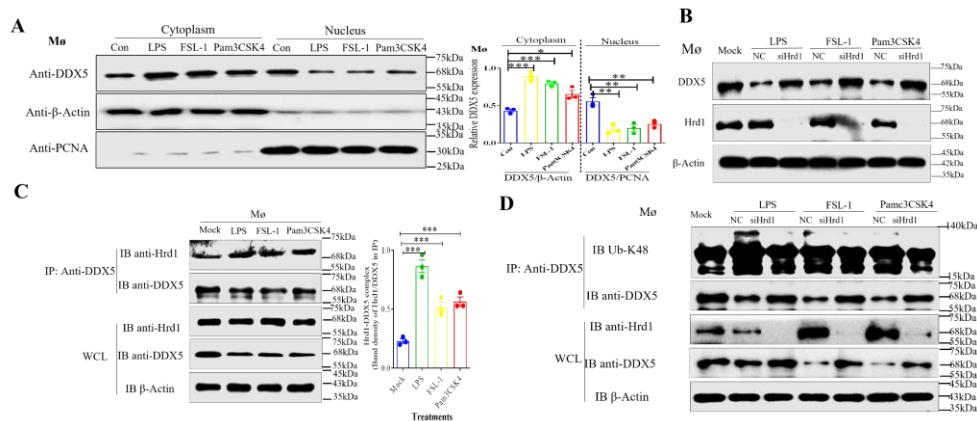
A: K48-linked ubiquitination of DDX5 in MEFs treated with LPS, FSL-1, and Pam3CSK4.

B: K48-linked ubiquitination of DDX5 in Mø treated with LPS, FSL-1, and Pam3CSK4.



Appendix Figure S3. Hrd1 pull-down assay in MEFs, related to Figure 3.

MEFs were seeded and cultured on 60-mm dishes for 24 h; then, the cells were lysed with NP40 lysis buffer containing protease inhibitor cocktail (Roche). Lysates were immunoprecipitated with a Rabbit anti-Hrd1 antibody or Rabbit IgG in conjunction with protein G/A-magnetic beads. Immunoprecipitated Hrd1 and its associated proteins from MEFs were then resolved on a PAGE gel for the indicated time. Different bands were analyzed by LC/MS. Arrow indicates the band of DDX5 protein detected by LC/MS.



Appendix Figure S4. DDX5 is degraded in mouse macrophages by Hrd1 triggered by TLR2/4 agonists, related to Figure 3.

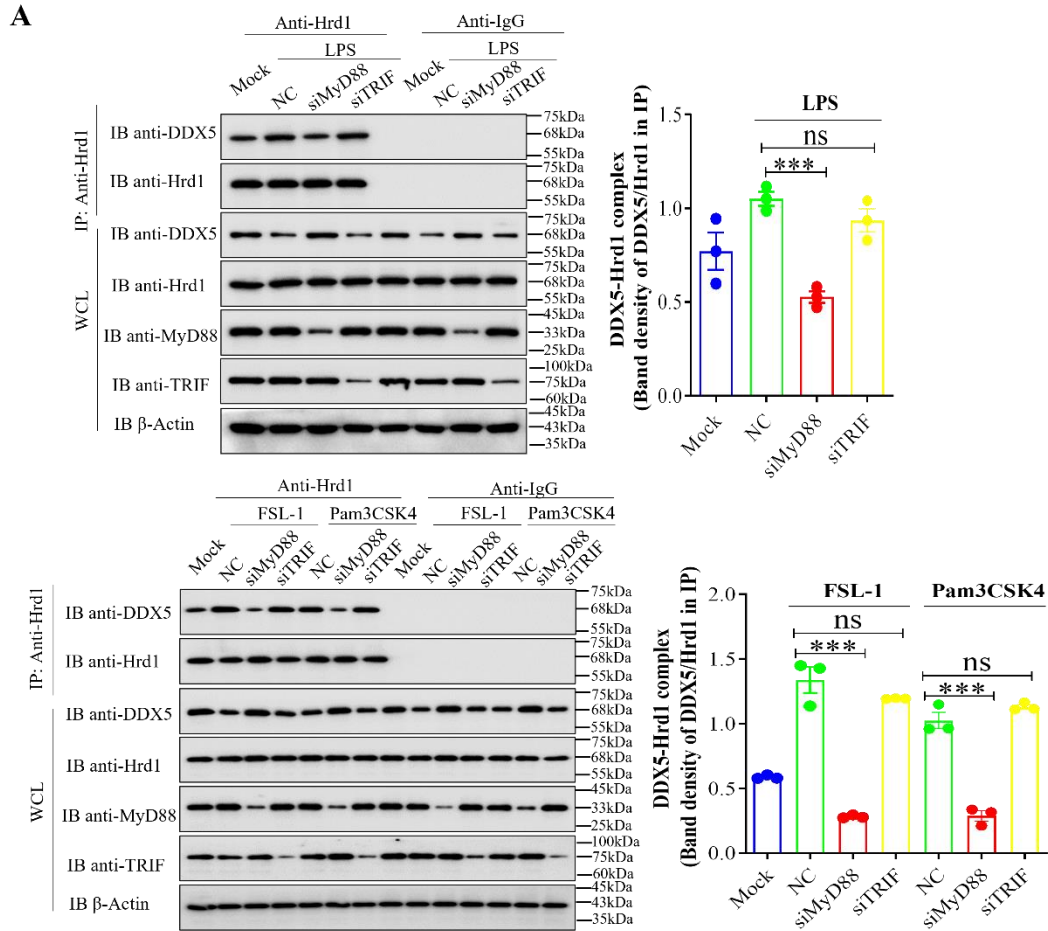
A: Redistribution of DDX5 after treatment of TLR2/TLR4 agonists. Mouse macrophages (Mø) were treated with LPS, FSL-1, and Pam3CSK4 respectively, harvested to extract the nuclear and cytoplasmic protein for western blot assay to detect the expression of DDX5. β-Actin acted as a cytoplasmic reference control, and PCNA was a nuclear reference control. The expression of cytoplasmic DDX5 was quantified by the relative intensity of DDX5/β-Actin, and the expression of nuclear DDX5 was quantified by the relative intensity of DDX5/PCNA. The band intensity was measured by Image J software.

B: DDX5 level in Hrd1 knockdown Mø after treatment with LPS, FSL-1, and Pam3CSK4; NC served as the siRNA negative control.

C: Interaction between Hrd1 and DDX5 in Mø after treatment with LPS, FSL-1, and Pam3CSK4 by Co-IP with an anti-DDX5 antibody. The interaction of Hrd1 and DDX5 was quantified by the band density of Hrd1/DDX5 in the IP system; the band density was measured by Image J software.

D: The K48-linked ubiquitin modification of DDX5 in Hrd1 knockdown Mø after treatment with LPS, FSL-1, and Pam3CSK4; NC served as the siRNA negative control.

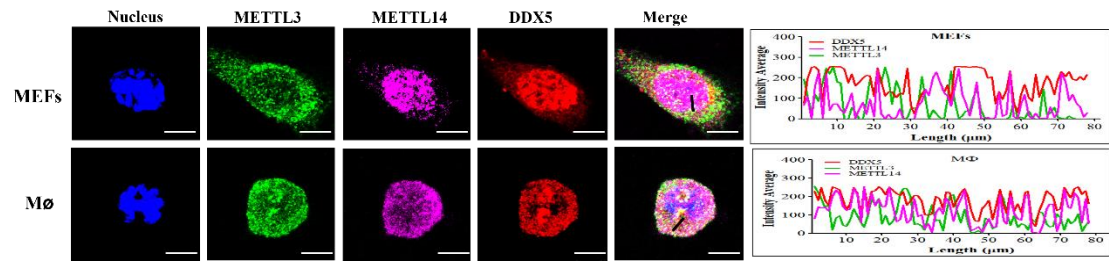
Data information: In (A, C), all data are represented as the mean ± SEM of three biologically independent samples. “ns” indicates no significant difference, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ (Student’s t-test).



Appendix Figure S5. The recruitment of DDX5 to Hrd1 upon TLR stimulation was MyD88-dependent pathway, related to Figure 3.

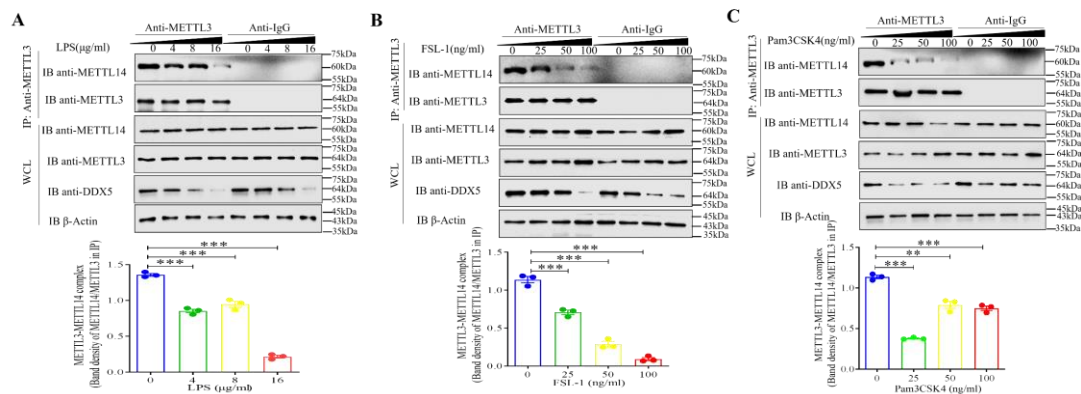
MyD88 and TRIF was knocked down in MEFs followed by treatment with LPS (A), and FSL-1 or Pam3CSK4 (B). NC served as the siRNA negative control. The interaction of DDX5 and Hrd1 was examined by Co-IP, and quantified by the relative density of DDX5/Hrd1. The band density was measured by Image J software.

Data information: In (A-B), all data are represented as the mean $\pm$ 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).



Appendix Figure S6. Co-localization of DDX5, METTL3, and METTL14 in MEFs or mouse macrophages, related to Figure 4.

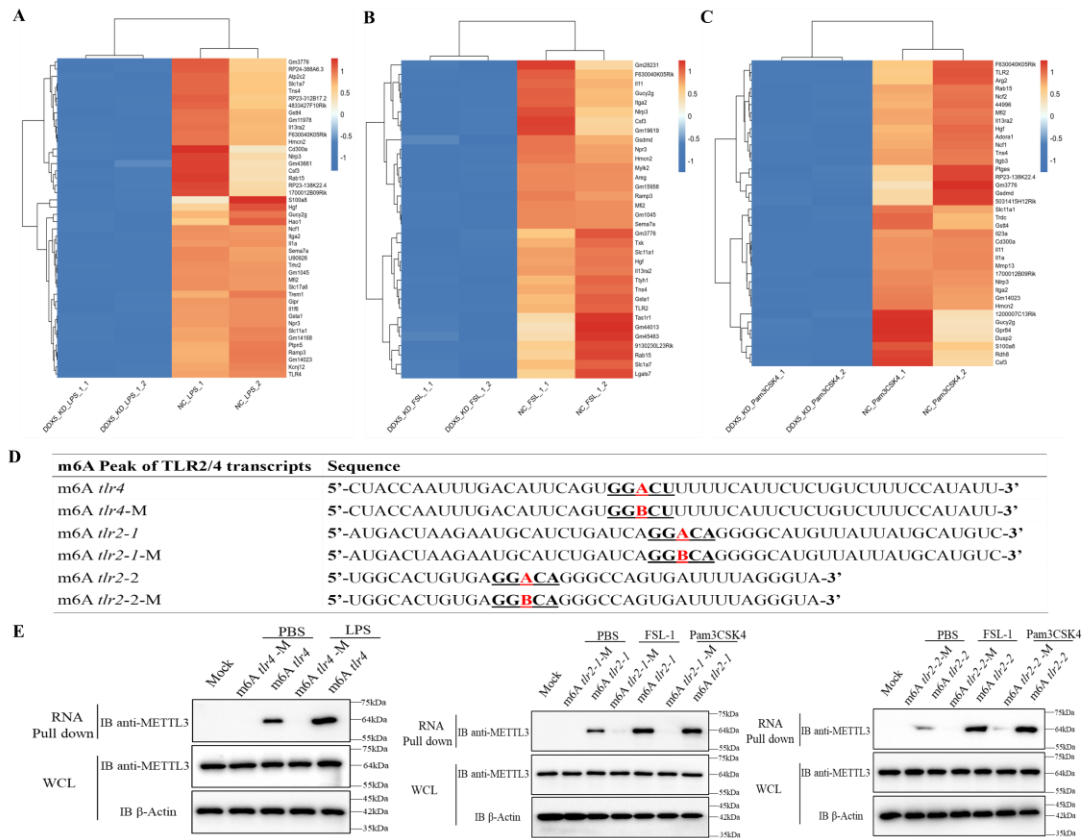
The MEFs or mouse macrophages (Mφ) were seeded on coverslips in 12-well plates and cultured for 18–24 h, then fixed with 4% paraformaldehyde and stained with anti-METTL3, anti-DDX5 and anti-METTL14 before confocal microscopy. Right panels show the pixel intensity of green (METTL3), purple (METTL14) and red (DDX5) from the black line in merge image. Scale bars: 7.5 μm.



Appendix Figure S7. The interplay of METTL3 and METTL14 in MEFs after treatment with TLR2/TLR4 agonists, related to Figure EV2.

Interaction between METTL3 and METTL14 in MEFs after treatment with different concentrations of LPS (0, 4, 8, 16 µg/ml) (A), FSL-1 (0, 25, 50, 100 ng/ml) (B), and Pam3CSK4 (0, 25, 50, 100 ng/ml) (C) by Co-IP with an anti-METTL3 antibody. The interaction of METTL3 and METTL14 was quantified by the band intensity of METTL14/METTL3 in the IP system; the band intensity was measured by Image J software.

Data information: In (A-C), all data are represented as the mean ± SEM of three biologically independent samples. **p < 0.01 and ***p < 0.001 (Student's t-test).

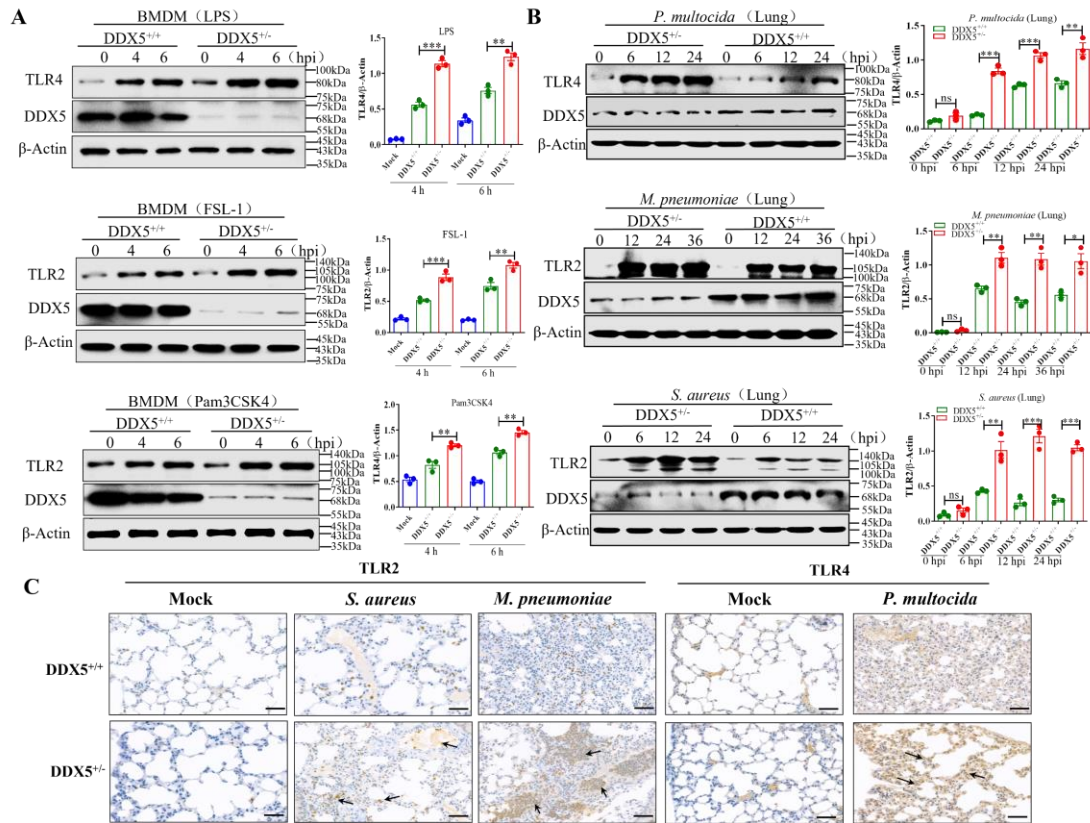


Appendix Figure S8. The analysis of m6A modification of TLR2/4 transcripts based on the MeRIP-seq, related to Figure EV3.

A-C: Heatmap of differential m6A modified transcripts of DDX5 knockdown (KD) MEFs after LPS (A), FSL-1 (B) and Pam3CSK4 (C) treatment. Differential m6A modified transcripts were generated from MeRIP-seq and RNA-seq data ($\log_{2}FC \geq 2.5$).

D: The sequence of m6A peak of TLR2/4 transcripts and its mutants. The sequence of m6A peak of TLR2/4 transcripts were required based on IGV assays of MeRIP-Seq. The underline indicated the m6A motif “RRACH”, the red A was the m6A site on TLR2/4, the red B (G/C/U) in mutant was replaced with red A of m6A site on TLR2/4. The black arrow indicated TLR2/4 transcripts.

E: RNA Pull down of m6A peak of TLR2/4 transcripts and its mutants with METTL3 in MEFs. The Biotin labeled m6A peak of TLR2/4 transcripts and its mutants were transfected into MEFs, then treated with TLR2/4 agonists, respectively. MEFs were lysed for RNA Pull down to detect the binding of m6A peak of TLR2/4 transcripts with mutants or METTL3. β -Actin was used as a reference control.



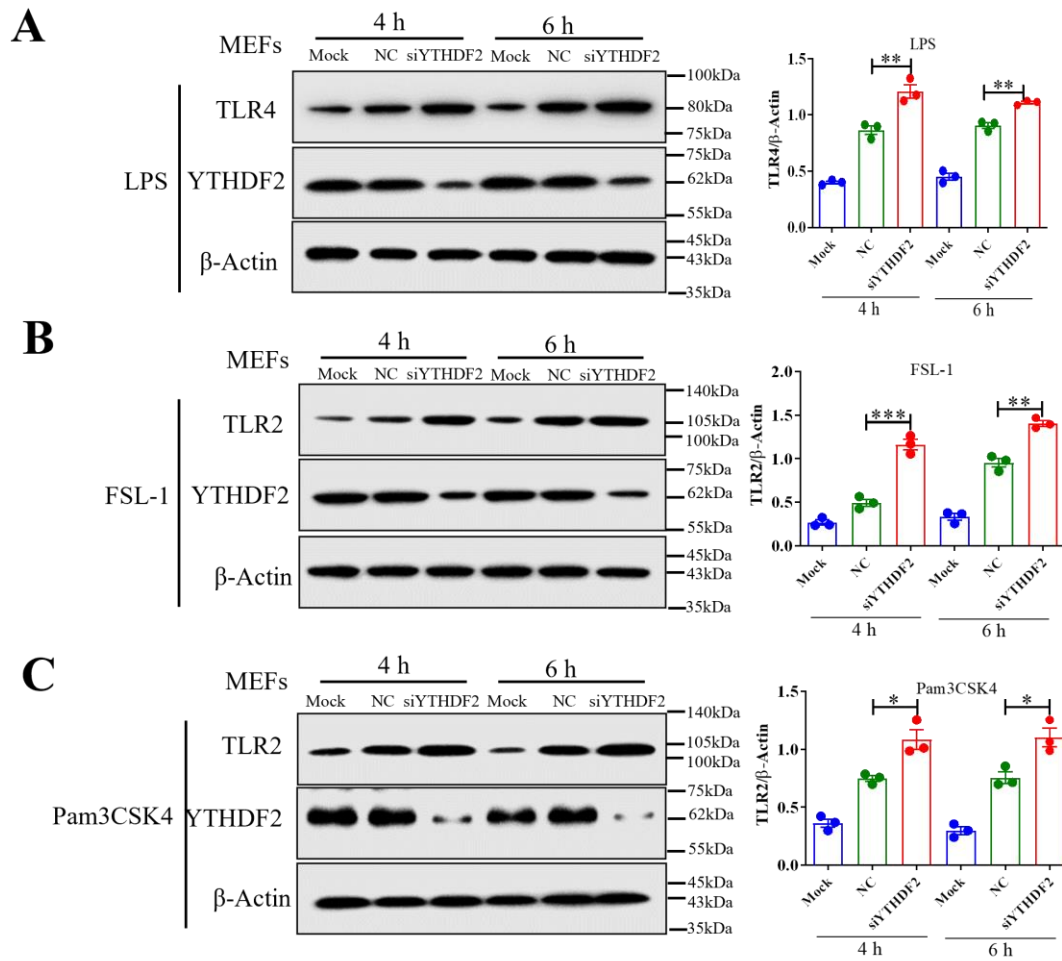
Appendix Figure S9. Expression of TLR4/TLR2 in primary BMDM or lungs of DDX5^{+/+} or DDX5^{+/-} mice, related to Figure 5.

A: Expression of TLR4, TLR2, DDX5, and β-Actin in primary DDX5^{+/+} BMDM or DDX5^{+/-} BMDM treated with LPS, FSL-1 or Pam3CSK4 for 4 or 6 h.

B: Expression of TLR4, TLR2, DDX5, and β-Actin in the lungs of DDX5^{+/+} or DDX5^{+/-} mice infected with *P. multocida* for 6, 12, and 24 h, *M. pneumoniae* for 12, 24, and 36 h, or *S. aureus* for 6, 12, and 24 h. 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: Immunohistochemistry analysis of TLR4 in the lungs of DDX5^{+/+} or DDX5^{+/-} mice infected with *P. multocida* for 12 h, and of TLR2 in the lungs of DDX5^{+/+} or DDX5^{+/-} mice infected with *S. aureus* for 12 h and *M. pneumoniae* for 24 h. Scale bars, 50 μm. The black arrows indicated strong positive signals.

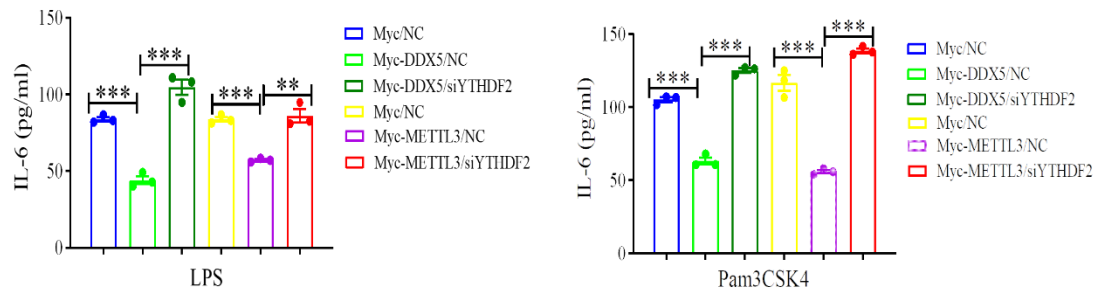
Data information: In (A-B), all data are represented as the mean ± SEM of three biologically independent samples. “ns” indicates no significant difference, *p < 0.05, **p < 0.01, and ***p < 0.001 (Student’s *t*-test).



Appendix Figure S10. TLR2/TLR4 protein level in YTHDF2-deficient MEFs treated with TLR2/4 agonists, related to Figure 6.

A-C: TLR4 protein level in NC and siYTHDF2 MEFs treated with LPS for 4 or 6 h (A), or TLR2 protein level in NC and siYTHDF2 MEFs treated with FSL-1 (B) or Pam3CSK4 (C) for 4 or 6 h. 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.

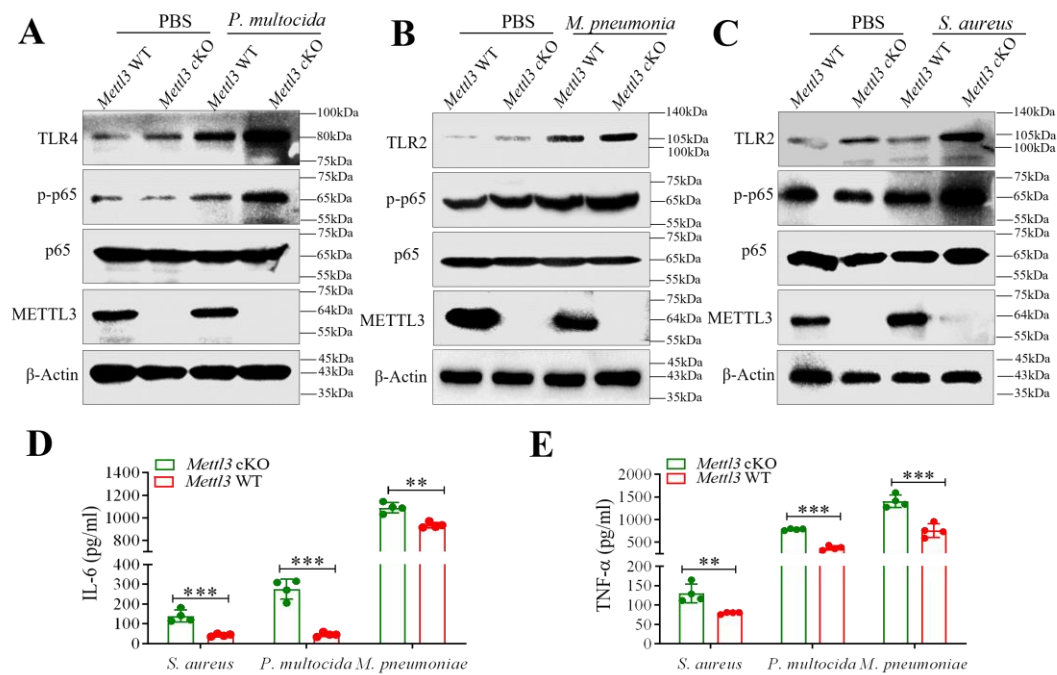
Data information: In (A-C), all data are represented as the mean $\pm$ SEM of three biologically independent samples. “ns” indicates no significant difference, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ (Student’s t -test).



Appendix Figure S11. IL-6 production in Myc-DDX5/Myc-METTL3 overexpressed MEFs with knockdowned-YTHDF2 treated with LPS, and Pam3CSK4, related to Figure 6.

Data information: 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).

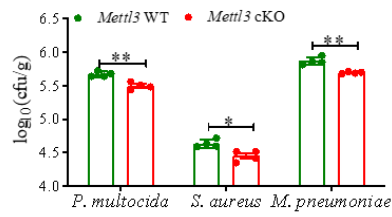


Appendix Figure S12. The expression of TLR4/2 and inflammatory cytokines in primary *Mettl3* WT or *Mettl3* cKO BMDM infected with pathogenic bacteria, related to Figure 7.

A-C: Expression of TLR4, p-p65, DDX5, and β -actin in primary *Mettl3* WT BMDM or *Mettl3* cKO BMDM infected with *P. multocida* for 12 h, *M. pneumoniae* for 24 h (B) or *S. aureus* for 12 h (C), respectively.

D, E: IL-6 (D) or TNF- α (E) production in primary *Mettl3* WT BMDM or *Mettl3* cKO BMDM infected with *P. multocida*, *S. aureus*, or *M. pneumoniae*, respectively (n=4).

Data information: In (D-E), all data are represented as the mean $\pm$ SEM of three biologically independent samples. *p < 0.05, **p < 0.01, and ***p < 0.001 (Student's *t*-test).



Appendix Figure S13. The bacteria loading of *P. multocida*, *S. aureus*, or *M. pneumoniae* was measured in the lungs of *Mettl3* WT or *Mettl3* cKO mice (n=4), related to Figure 7.

Data information: All data are represented as the mean $\pm$ SEM of three biologically independent samples.

*p < 0.05, and **p < 0.01 (Student's *t*-test).