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Supplemental information

m^6A mRNA methylation-directed myeloid cell activation controls progression of NAFLD and obesity

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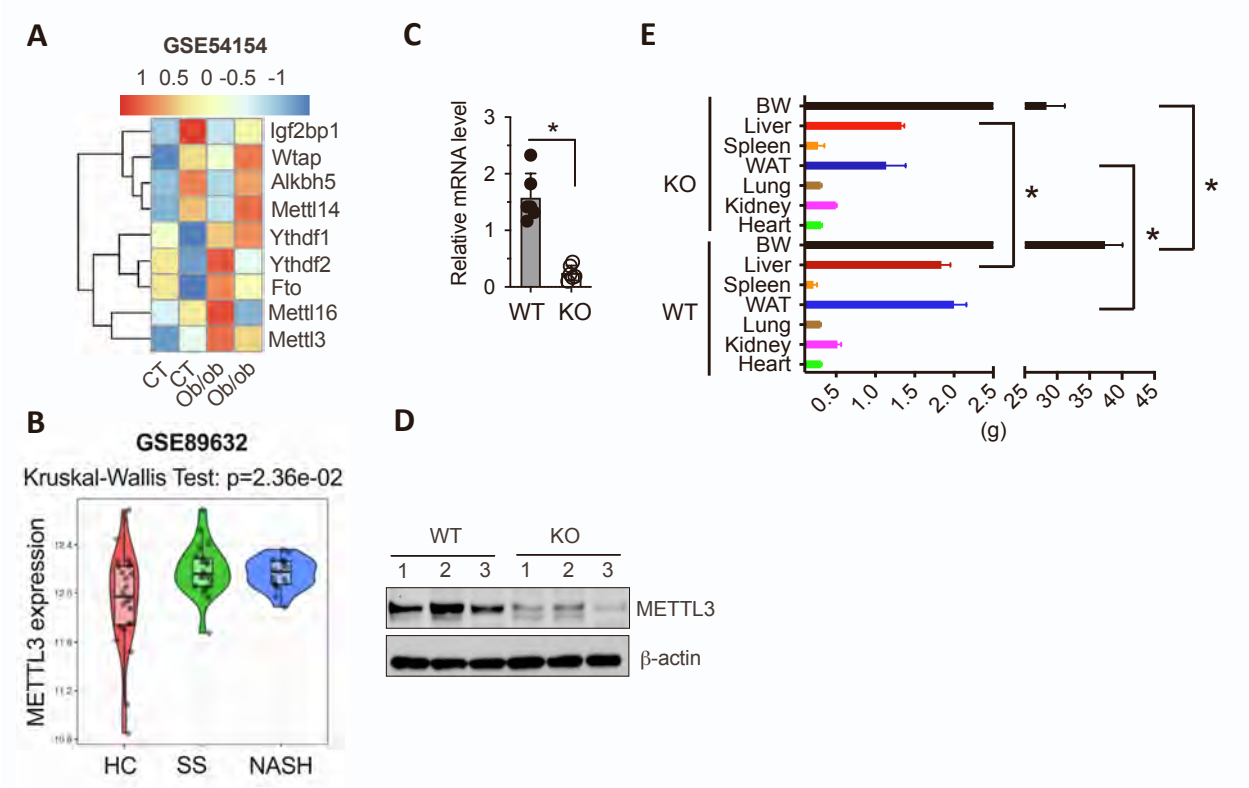
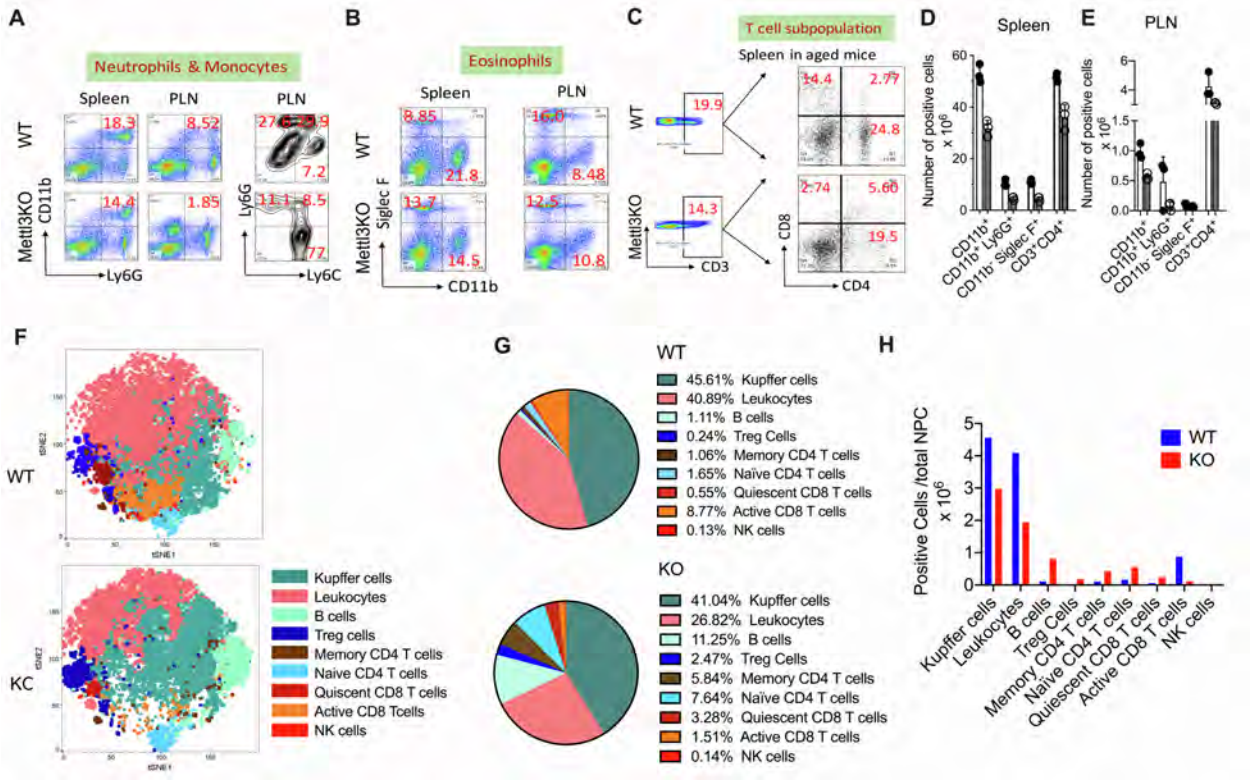
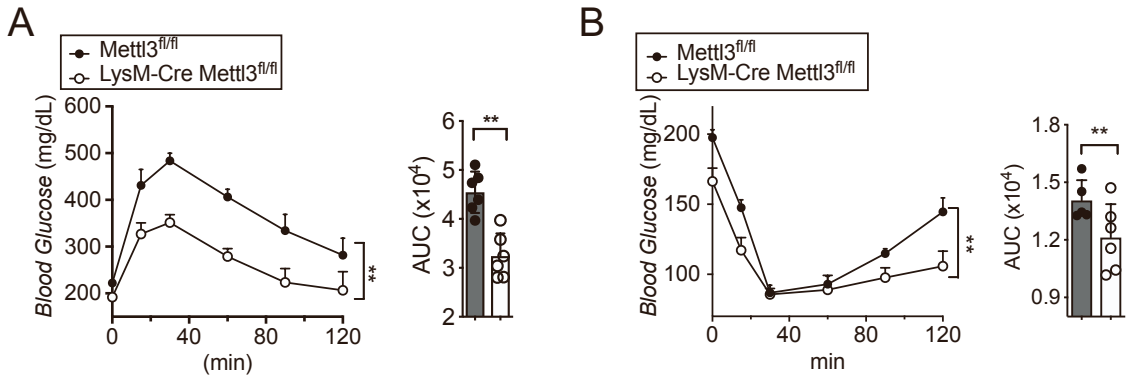


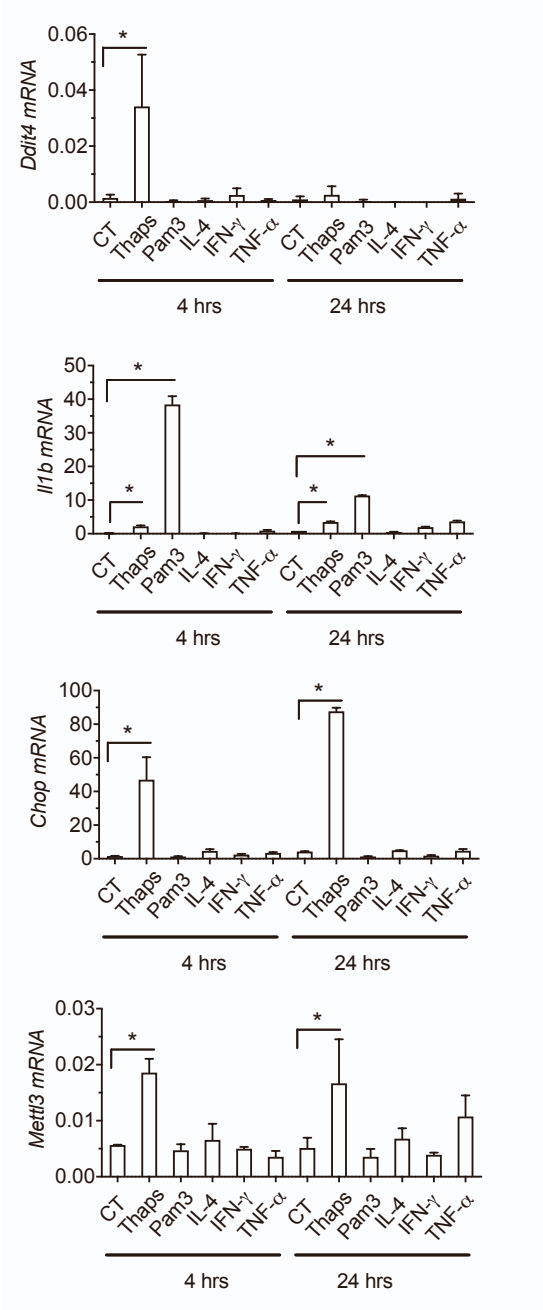
Supplementary Figure 1: Targeting m^{6}A in macrophages. Related to Figure1 & 2. (A, B) m^{6}A genes are elevated in BMDMs from T2D Ob/Ob mice and in liver from NAFLD patients. Related to Figure 1 & 2. (**A**). Re-analyses the expression pattern of m^{6}A genes using the RNA-sequencing dataset (GSE54154) from BMDMs collected from T2D Ob/Ob mice and controls. (**B**) Re-analysis the Mettl3 expression in liver tissue using one NAFLD dataset (GSE89632) including 20 patients with simple steatosis (SS), 19 with nonalcoholic steatohepatitis (NASH), and 24 healthy controls (HC). **(C-E) Generation of *Mettl3* LysM-Cre conditional knockout mice. Related to Figure 1.** Mettl3 $^{flox/flox}$ mice were crossed with LysMcre to generate LysM-Cre$^{+/-}$ Mettl3 $^{flox/flo\,x}$ (KO) and Mettl3 $^{flox/flox}$ littermates without LysM-Cre gene (WT). **(C)** mRNA levels of Mettl3 gene were measured by qRT-PCR in BMDMs collected from KO and WT mice. Data represent mean ± SD (n =6). * $P < 0.01$ by unpaired t test. **(D)** Protein levels of METTL3 were analyzed by Western Blot in BMDMs from KO and WT mice (n=3). (**E**) Changes of organ and tissue weight from aged KO and WT mice. Organs and tissues as indicated were collected and weighted from 46 weeks of KO and WT mice Data represent mean ± SD (n =9). * $P < 0.01$ by two-tailed Student's *t* test or two-way ANOVA.



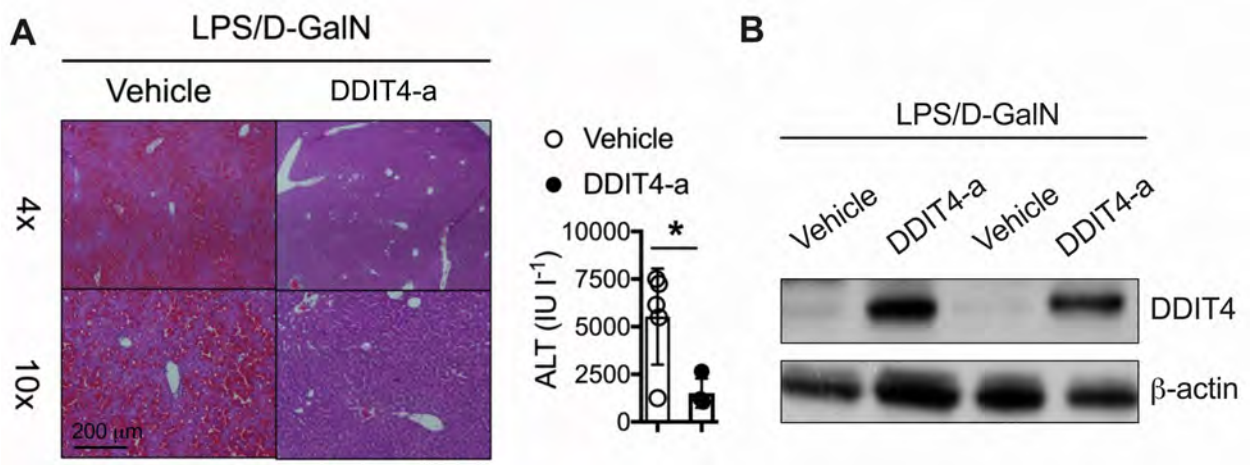
Supplementary Figure 2: Changes of immune cell populations from aged KO and WT mice. Related to Figure 1. Percentages of myeloid cell subtypes of $CD11b^+/Ly6G^+$ (neutrophils) and $CD11^+/Ly6C^+$(monocytes) (**A**), and CD11b+/ Siglec F^+ (Eosinophils) (**B**), and T cell populations of $CD3^+$ (total T cells), $CD3^+$ CD4+ (CD4 T cells) and $CD3^+$ $CD8^+$ (CD8 T cells) (**C**) were analyzed by FACS. The total cells of subpopulations in spleen (**D**) and PLN (**E**) were shown. Representative flow from 3 repeats was shown. (**F- H**) Immune cell subpopulations in liver non-parenchymal liver cells from aged KO and WT littermate control mice were analyzed by mass cytometry (CyTOF). (**F**) Density dot plot from CyTOF showing clustered subpopulations. (**G**) Proportion of clustered subpopulations. (**H**) Total cells of clustered subpopulations. Results were from two repeats.



Supplementary Figure 3: METTL3 deficiency in myeloid cells enhances insulin sensitivity from diet- induced NAFLD and obesity. Related to Figure 2. The 10-week-old METTL3 KO and WT littermate mice were fed with high fat diet for 12 weeks (n=6). The mice were fasted over 5 hours, and glucose tolerance test (GTT) (**A**) and insulin tolerance test (ITT) (**B**) were performed. Mice were in intraperitoneally injected glucose (2g/kg body weight) (**A**), or insulin (0.9U/kg body weight), and the glucose levels were measured using Accu-Check inform II system. Glucose area under the curve (AUC) was calculated. Data represent mean ± SD (n =6). ** $P < 0.01$. For GTT and ITT studies, two-way ANOVA with multiple comparison was used for statistical analysis. AUC was calculated for each mouse and evaluated by two-tailed unpaired Student's t test for statistical differences between two genotypes.



Supplementary Figure 4: ER stress induces DDIT4 gene expression in BMDMs. Related to Figure 4. BMDMs were treated with Thapsigargin (10uM), Pam3CSK4 (100 ng/ml), IL-4 (20 ng/ml), IFN-γ (20 ng/ml) and TNF-α (50ng/ml) for the indicated time-course. The total RNA was isolated, and qRT-PCR was performed using specific primers as indicated. The gene expression level was normalized with β-actin. Data represent mean ± SD (n =6). * $P < 0.05$ by two-tailed Student's *t* test or two-way ANOVA.



Supplementary Figure 5: DDIT4 activation suppresses acute inflammatory liver injury. Related to Figure 5. Wild type mice were injected with DDIT4 activator (DDIT4-a) (2 mg/kg) by I.P followed LPS/D-GalN 1 h later and examined after 6 h. (**A**) Reduction of hemorrhage, cellular necrosis and serum alanine transaminase (ALT) were shown in DDIT4-a treated mice. (**B**) DDIT4 protein levels in liver tissue by DDIT4-a treatment by Western blot. Data represent mean ± SD (n =5 mice). * $P < 0.05$ by unpaired t test.