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Supplemental information

METTL14-dependent m⁶A modification controls

iNKT cell development and function

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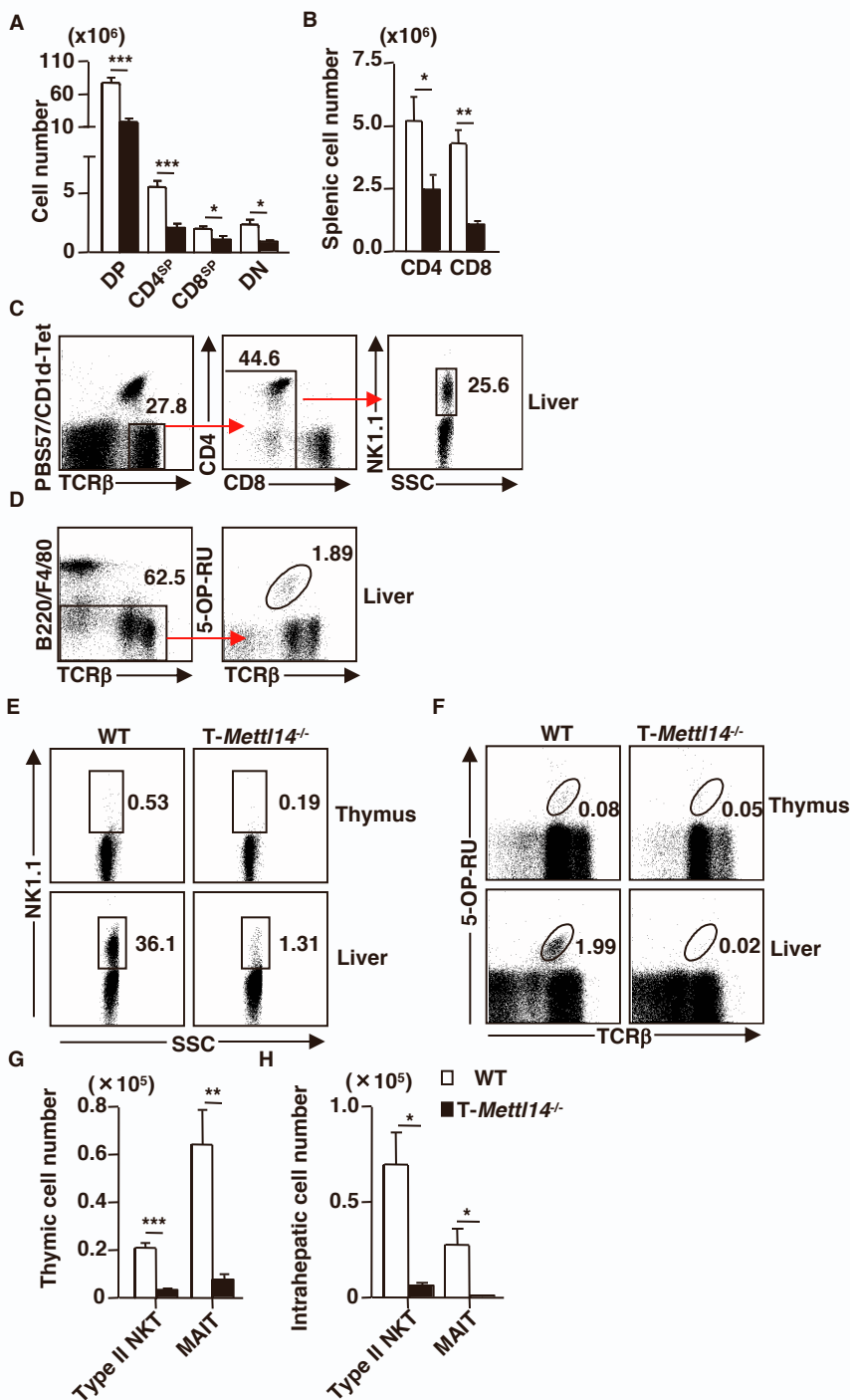


Figure S1. *Mettl14*-deficiency impaired development of various T cell subsets in the thymus and peripheral. Related to Figure 1. (A) Cell number of DP, CD4^{SP}, CD8^{SP} and DN in thymus. **(B)** Cell number of CD4⁺ and CD8⁺ T cells in spleen. **(C)** Gating strategy of type II NKT cells in the liver of B6 mice. **(D)** Gating strategy of MAIT cells. **(E)** Representative FACS plot of type II NKT cells in thymus and liver. **(F)** Representative plot of MAIT cells in thymus and liver. Cell number of type II NKT cells and MAIT cells in thymus **(G)** and liver **(H)** (n=6-7). SEM is shown. **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

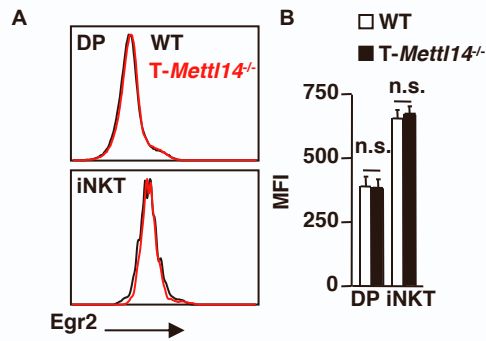


Figure S2. The expression of Egr2 was not altered in T-*Mettl14*^{-/-} mice. Related to Figure 3. (A) Histogram of Egr2 expression in DP thymocytes and thymic iNKT in T-*Mettl14*^{-/-} mice. **(B)** Bar graph of MFI of Egr2 in DP thymocytes and thymic iNKT in T-*Mettl14*^{-/-} mice (n=3). SEM is shown. n.s., not significant.

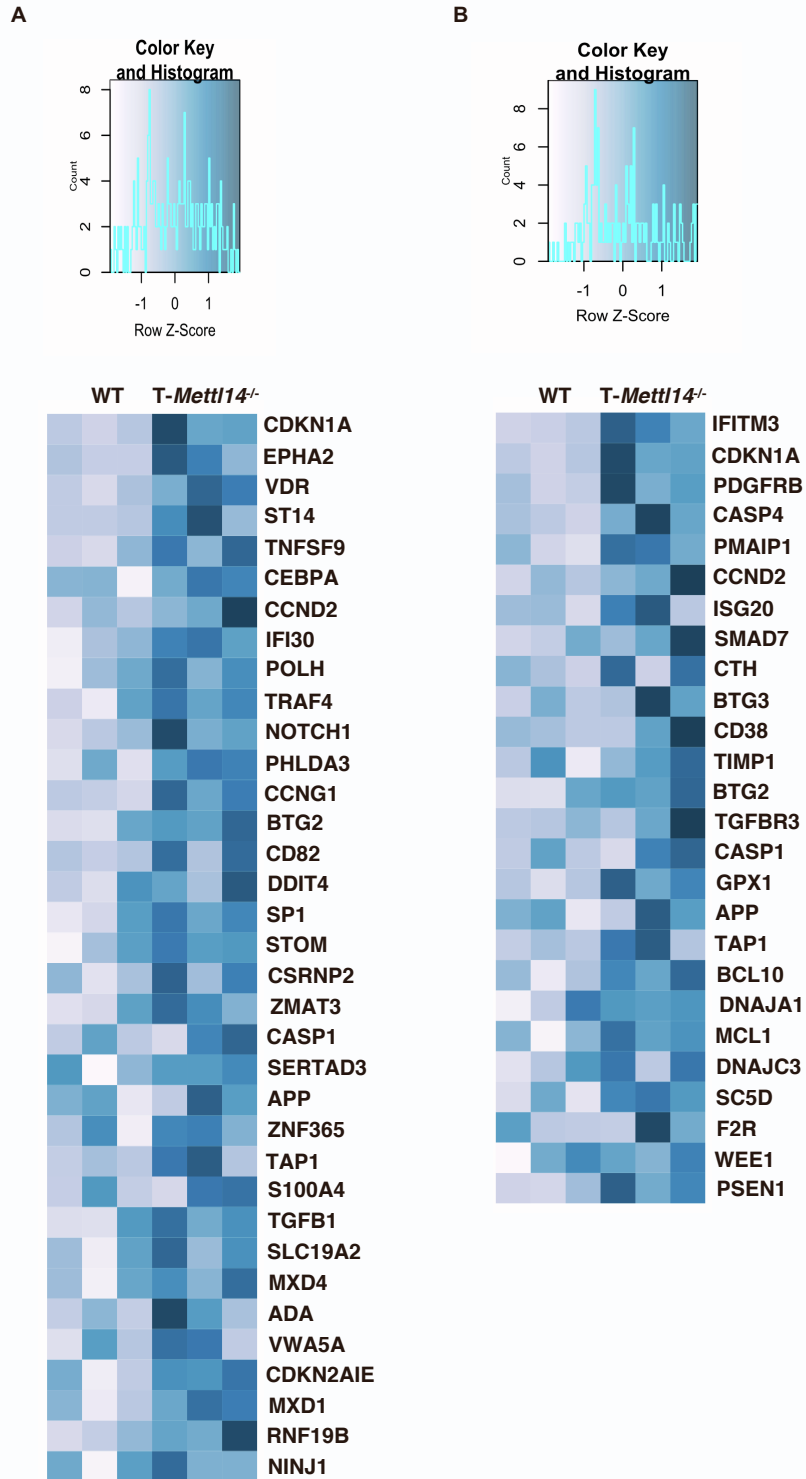


Figure S3. Heat map of GSEA core enriched genes. Related to Figure 4. (A) P53 pathway. (B) Apoptosis pathway.

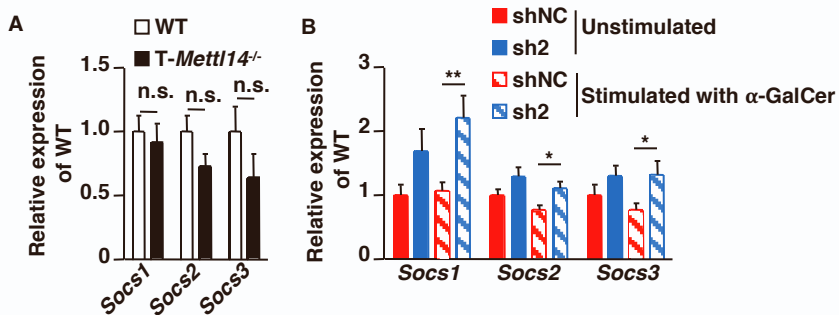


Figure S4. Expression of *Socs* family genes in T-*Mettl14*^{-/-} DP thymocytes and *Mettl14*^{KD} DN32.D3 NKT cell hybridomas. Related to Figure 6 and 7.

(A) The expression of *Socs1*, *Socs2* and *Socs3* was not altered in T-*Mettl14*^{-/-} DP thymocytes. (n=6) (B) Increased expression of *Socs1*, *Socs2* and *Socs3* in *Mettl14*^{KD} DN32.D3 (n=5-6). SEM is shown. **P* < 0.05; ***P* < 0.01.

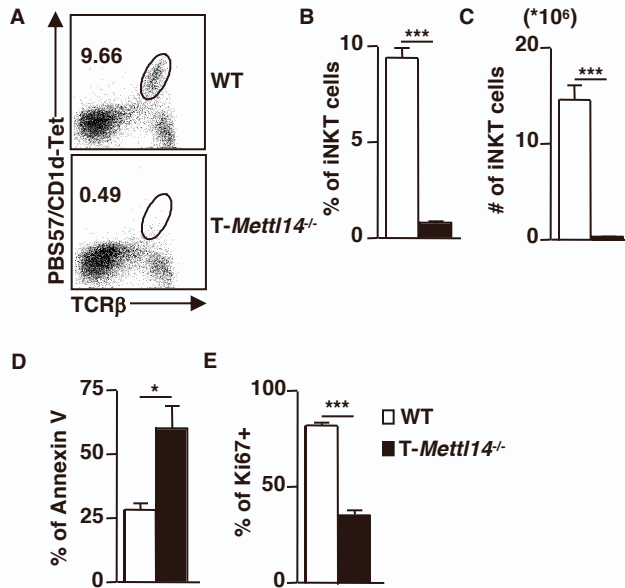


Figure S5. Expansion of splenic iNKT cells was impaired in T-Mettl14^{-/-} mice after *in vivo* α -GalCer injection. Related to Figure 7. (A) Representative dot plots of splenic iNKT cells in WT and T-Mettl14^{-/-} mice at D3 after injection with α -GalCer (IV). (B and C) Quantification of percentage and absolute cell number of splenic iNKT cells in WT and T-Mettl14^{-/-} mice. (D) Percentage of Annexin V⁺ in splenic iNKT cells in WT and T-Mettl14^{-/-} mice. (E) Percentage of Ki67⁺ in splenic iNKT cells. (n=4). SEM is shown. **P* < 0.05; **P* < 0.001.**

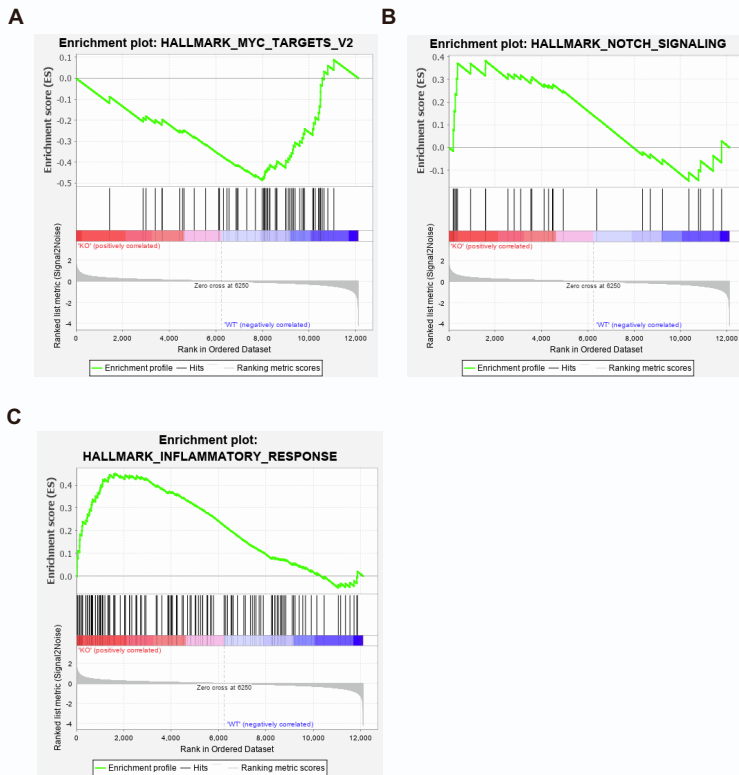


Figure S6. GSEA analysis of RNaseq data from WT and *T-Mettl14*^{-/-} DP thymocytes. Related to Figure 4. (A) Myc signaling pathway. (B) Notch signaling pathway. (C) Inflammation response.