Supplementary Information for

RNA m⁶A demethylase ALKBH5 regulates the development of γδ T cells

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**SI Appendix, Fig. S1.** ALKBH5 is expressed in a wide range of immune cells. (A) Normalized expression value of ALKBH5 across different immune cells. Data is acquired from Immunological Genome Project (www.immgen.org). (B) Schematic representation of the targeting strategy used to flank exon 1 of the Alkbh5 loci with loxP sites (top) and excision with Cre-recombinase (bottom). (C) Mouse models employed to specifically knock-out ALKBH5 in lymphocyte populations. (D) Quantitative PCR (qPCR) analysis of ALKBH5 gene expression level in thymocytes isolated from 4 pairs of Alkbh5<sup>fl/fl</sup> Lck<sup>+</sup> and Alkbh5<sup>fl/fl</sup> cohoused mice (n = 4). Data represent mean ± s.d.; paired t test was used for statistical analysis. **P < 0.01.
SI Appendix, Fig. S2. ALKBH5 deficiency leads to expanded γδ T cell population in the periphery. (A) Flow cytometric analysis of lymphocytes isolated from indicated lymphoid organs and tissues (pLN, peripheral lymph node; mLN, mesenteric lymph node) of Alkbh5<sup>fl/fl</sup> Lck<sup>+</sup> and Alkbh5<sup>fl/fl</sup> mice. (B) Statistical analysis of cell numbers in (A) is reported. Unpaired t test was used for statistical analysis. Each dot represents one mouse (WT, n = 7; KO, n = 6). Data represent one out of three independent experiments (mean ± s.d.). **P < 0.01, ***P < 0.001, n.s., not significant.
SI Appendix, Fig. S3. Loss of ALKBH5 has minimum effects on αβ T cell development. (A, C and E) Flow cytometric analysis of CD4+ and CD8+ T cells isolated from the spleen (A), pLN (C) and mLN (E) of Alkbh5fl/fl Lck+ and Alkbh5fl/fl mice. The right panel reports the statistical analysis of frequencies for CD4+ and CD8+ T cells on the left. Unpaired t test was used for statistical analysis. Each dot represents one mouse (WT, n = 7; KO, n = 6). Data represent one out of three independent experiments (mean ± s.d.). (B, D and F) Flow cytometric analysis of naïve and memory T cells isolated from the spleen (B), pLN (D) and mLN (F) of Alkbh5fl/fl Lck+ and Alkbh5fl/fl mice. The right panel reports the statistical analysis of frequencies for effector memory (CD44), naïve (CD62L), central memory (DP) and double-negative (DN) cells on the left. Unpaired t test was used for statistical analysis. Each dot represents one mouse (WT, n = 7; KO, n = 6). Data represent one out of three independent experiments (mean ± s.d.). *P < 0.05, **P < 0.01, n.s., not significant.
SI Appendix, Fig. S4. Loss of ALKBH5 has minimum effects on αβ T cell development (continued). (A, C and E) Statistical analysis of cell numbers for CD4+ and CD8+ T cells isolated from the spleen (A), pLN (C) and mLN (E) of Alkbh5f/f Lck+ and Alkbh5f/f mice is reported, correlated with SI Appendix, Fig. S3 (A, C and E), respectively. Unpaired t test was used for statistical analysis. Each dot represents one mouse (WT, n = 7; KO, n = 6). Data represent one out of three independent experiments (mean ± s.d.). (B, D and F) Statistical analysis of cell numbers for naïve and memory T cells isolated from the spleen (B), pLN (D) and mLN (F) of Alkbh5f/f Lck+ and Alkbh5f/f is reported, correlated with SI Appendix, Fig. S3 (B, D and F), respectively. Unpaired t test was used for statistical analysis. Each dot represents one mouse (WT, n = 7; KO, n = 6). Data represent one out of three independent experiments (mean ± s.d.). *P < 0.05, **P < 0.01, n.s., not significant.
**SI Appendix, Fig. S5.** Loss of ALKBH5 doesn’t affect the proportion and balance of αβ T cell subsets in CD4-Cre stain. (A) Statistical analysis of frequencies for αβ/γδ T cells in thymus from Alkbh5f/f CD4+ and Alkbh5f/f mice (WT, n = 4; KO, n = 5). (B) Statistical analysis of frequencies showing CD4+, CD8+, double positive (DP) and double negative (DN) populations isolated from thymus of Alkbh5f/f CD4+ and Alkbh5f/f mice (WT, n = 4; KO, n = 5). (C) Statistical analysis of frequencies showing thymic lymphocytes at different DN stages. Cells were isolated from the thymus of either Alkbh5f/f CD4+ and Alkbh5f/f mice (WT, n = 4; KO, n = 5). (D) Statistical analysis of frequencies showing IFN-γ and IL-17A production in αβ T cells. Cells were isolated from the thymus of either Alkbh5f/f CD4+ and Alkbh5f/f mice (WT, n = 5; KO, n = 5). (E) Weight loss of Alkbh5f/f (n = 6) and Alkbh5f/f CD4+ (n = 6) mice infected with S. typhimurium. (F) Survival curve for S. typhimurium-infected Alkbh5f/f (n = 6) or Alkbh5f/f CD4+ (n = 6) mice. Log rank test was used for analysis. Unpaired t test was used for statistical analysis. (G) S. typhimurium Colony-forming Units (CFUs)/g of feces. Data represent one out of three independent experiments. Unpaired t test was used for statistical analysis for (A), (B), (C), (D) and (E). Log rank test was used for (F). Mann-Whitney test was used for (G). Each dot represents one mouse. Data represent one out of three independent experiments (mean ± s.d.). n.s., not significant.
**SI Appendix, Fig. S6.** Different subsets of mature γδ T cells are all expanded in Alkbh5<sup>f/f</sup> Lck<sup>+</sup> mice. (A) Flow cytometric analysis of three mature γδ T cell subsets (CD8αβ: CD8α<sup>+</sup>, CD8β<sup>+</sup>, CD8αα: CD8α<sup>+</sup>, CD8β<sup>+</sup>, CD8β<sup>+</sup>; CD8αα<sup>+</sup>, CD8β<sup>-</sup>) isolated from indicated organs of either Alkbh5<sup>f/f</sup> Lck<sup>+</sup> or Alkbh5<sup>f/f</sup> mice. (B and C) Statistical analysis of frequencies (B) and cell numbers (C) for three mature γδ T cell subsets in (A) is reported. Unpaired t test was used for statistical analysis. Each dot represents one mouse (WT, n = 7; KO, n = 5). Data represent one out of three independent experiments (mean ± s.d.). **P < 0.01, ***P < 0.001, ****P < 0.0001, n.s., not significant.
SI Appendix, Fig. S7. Loss of ALKBH5 has no impact on IFN-γ and IL-17 production, proliferation and apoptosis of mature γδ T cells. (A) Flow cytometric analysis of IFN-γ and IL-17A production in mature γδ T cells isolated from the thymus, spleen, pLN and mLN of Alkbh5f/f Lck+ and Alkbh5f/f mice. (B) Statistical analysis of the frequencies for IFN-γ/IL-17A-producing mature γδ T cells isolated from the thymus, spleen, pLN and mLN of Alkbh5f/f Lck+ (n = 7) and Alkbh5f/f (n = 7) mice. Unpaired t test was used for statistical analysis. Each dot represents one mouse. Data represent one out of three independent experiments (mean ± s.d.). (C) Flow cytometric analysis of Ki-67 expression in mature γδ T cells isolated from the thymus of Alkbh5f/f Lck+ and Alkbh5f/f mice. (D) Statistical analysis of Ki-67+ γδ T mature cells in thymus in (C) is reported (WT, n = 6; KO, n = 6). Unpaired t test was used for statistical analysis. Each dot represents one mouse. Data represent one out of three independent experiments (mean ± s.d.). (E) Flow cytometric analysis of thymic mature γδ T cells undergoing apoptosis of Alkbh5f/f Lck+ and Alkbh5f/f mice. (F) Frequency of apoptotic cells (Annexin V+) in (E) is reported (WT, n = 6; KO, n = 8). Unpaired t test was used for statistical analysis. Each dot represents one mouse. Data represent one out of three independent experiments (mean ± s.d.). n.s., not significant.
SI Appendix, Fig. S8. Loss of ALKBH5 has no impact on IFN-γ or IL-17 production of mature αβ/γδ T cells. (A) Statistical analysis of the frequencies of IFN-γ-/IL-17A-/IL-4-producing αβ T cells isolated from the thymus of Alkbh5ff Lck+ (n = 7) and Alkbh5ff (n = 7) mice. (B) Statistical analysis of the frequencies of IFN-γ-/IL-17A-/IL-4-producing αβ T cells isolated from the spleen of Alkbh5ff Lck+ (n = 7) and Alkbh5ff (n = 7) mice. (C) Statistical analysis of frequencies for IFN-γ-/IL-17A-producing mature γδ T cells isolated from Colon-IEL of Alkbh5ff Lck+ (n = 7) and Alkbh5ff (n = 6) mice. (D) Statistical analysis of frequencies for IFN-γ-/IL-17A-producing mature γδ T cells isolated from Colon-LPL of Alkbh5ff Lck+ (n = 7) and Alkbh5ff (n = 6) mice. Unpaired t test was used for statistical analysis. Each dot represents one mouse. Data represent one out of three independent experiments (mean ± s.d.). n.s., not significant.
**SI Appendix, Fig. S9.** ALKBH5 depletion induced expansion of γδ T cells that was not due to Lck-Cre toxicity. (A) Statistical analysis of frequencies for αβ/γδ T cells in the thymus from Alkbh5<sup>fl/fl</sup> Lck<sup>+</sup> and Lck<sup>+</sup> mice (WT, n = 4; KO, n = 5). (B) Statistical analysis of cell numbers in (A) is reported (WT, n = 4; KO, n = 5). (C) Statistical analysis of frequencies showing CD4<sup>+</sup>, CD8<sup>+</sup>, double positive (DP), and double negative (DN) populations isolated from the thymus of Alkbh5<sup>fl/fl</sup> Lck<sup>+</sup> and Lck<sup>+</sup> mice (WT, n = 4; KO, n = 5). (D) Statistical analysis of cell number for DN population in (C) is reported (WT, n = 4; KO, n = 5). (E) Statistical analysis of frequencies showing thymic lymphocytes at different DN stages. Cells were isolated from the thymus of either Alkbh5<sup>fl/fl</sup> Lck<sup>+</sup> or Lck<sup>+</sup> mice (WT, n = 4; KO, n = 5). (F) Statistical analysis of cell number for cells at DN2 and DN3 stages in (E) is reported (WT, n = 4; KO, n = 5). Unpaired t test was used for statistical analysis. Each dot represents one mouse. Data represent one out of three independent experiments (mean ± s.d.). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001, n.s., not significant.
SI Appendix, Fig. S10. Ratio of thymic γδ T cells subsets and their precursor subsets remain intact or increase slightly in Alkbh5f/f Lck* mice. (A, C and E) Flow cytometric analysis of immature TCRγ1.1+ γδ T cells at double negative (DN) stage (A), TCRγ1.1+ γδ T precursor cells (defined as CD73+ TCRγ1.1+ γδ T cells at DN stage) (C) and NKTγ1.1 precursor cells (defined as CD73+ CD24− NK1.1+ TCRγ1.1+ γδ T precursor cells) (E). The right panels report the statistical analysis of frequencies for the populations gated in the left panels. Unpaired t test was used for statistical analysis. Each dot represents one mouse (WT, n = 7; KO, n = 6). Data represent one out of three independent experiments (mean ± s.d.). (B, D and F) Flow cytometric analysis of immature TCRγ2+ γδ T cells at DN stage (B), TCRγ2+ γδ T precursor cells (defined as CD73+ TCRγ2+ γδ T cells at DN stage) (D) and NKTγ2 precursor cells (defined as CD73+ CD24− NK1.1+ TCRγ2+ γδ T precursor cells) (F). The right panels report the statistical analysis of frequencies for the populations gated in the left panels. Unpaired t test was used for statistical analysis. Each dot represents one mouse (WT, n = 7; KO, n = 6). Data represent one out of three independent experiments (mean ± s.d.). *P < 0.05, n.s., not significant.
SI Appendix, Fig. S11. All subsets of thymic mature γδ T cells are expanded in Alkbh5fr Lck+ mice based on TCRγ. (A) Flow cytometric analysis of thymic mature TCRγ1.1+ γδ T cells isolated from Alkbh5fr Lck+ and Alkbh5fr mice. (B and C) Statistical analysis of frequency (B) and cell number (C) for the gated population in (A) is reported. Unpaired t test was used for statistical analysis. Each dot represents one mouse (WT, n = 6; KO, n = 5). Data represent one out of three independent experiments (mean ± s.d.). (D) Flow cytometric analysis of thymic mature TCRγ2+ γδ T cells isolated from Alkbh5fr Lck+ and Alkbh5fr mice. (E and F) Statistical analysis of frequency (E) and cell number (F) for the gated population in (D) is reported. Unpaired t test was used for statistical analysis. Each dot represents one mouse (WT, n = 6; KO, n = 5). Data represent one out of three independent experiments (mean ± s.d.). (G) Flow cytometric analysis of thymic mature γδNKT cells isolated from Alkbh5fr Lck+ and Alkbh5fr mice. (H and I) Statistical analysis of frequency (H) and cell number (I) for the gated population in (G) is reported. Unpaired t test was used for statistical analysis. Each dot represents one mouse (WT, n = 6; KO, n = 5). Data represent one out of three independent experiments (mean ± s.d.). ***P < 0.001, ****P < 0.0001, n.s., not significant.
**Si Appendix, Fig. S12.** Transcriptomic changes in ALKBH5 deficient γδ T cell progenitors. (A) Numbers of genes that are up-regulated and down-regulated in ALKBH5 deficient γδ T cell progenitors. Differently expressed genes based on P-value (P < 0.05). (B) Gene Ontology (GO) enrichment analysis of biological process for up-regulated genes in ALKBH5-deficient γδ T cell precursors.
Appendix, Fig. S13. Transcriptomic changes in ALKBH5 deficient mature γδ T cells. (A) Numbers of genes that are up-regulated and down-regulated in ALKBH5 deficient mature γδ T cells. Differently expressed genes based one P-value (P < 0.05). (B) qPCR analysis of Jagged1 and Notch2 mRNAs expression in mature γδ T cells isolated from Alkbh5fl/fl Lck+ (n = 3) and Alkbh5fl/fl (n = 3) mice. (C) qPCR analysis of Jagged1 and Notch2 mRNAs expression in mature γδ T cells and γδ T cell progenitors isolated from wild-type C57BL/6N mice (n = 3). (D) Heatmap showing Jagged/Notch signaling-related genes expression across different samples. (E and F) Gene Ontology (GO) enrichment analysis of biological process for down-regulated (E) and up-regulated genes (F) in ALKBH5-deficient mature γδ T cells. Unpaired t test was used for statistical analysis. Data represent one out of three independent experiments (mean ± s.d.). *P < 0.05, **P < 0.01, n.s., not significant.
**SI Appendix, Fig. S14.** Jagged1 depletion induced expansion of γδ T cells. (A) Statistical analysis of frequencies showing CD4⁺, CD8⁺, double positive (DP), and double negative (DN) populations isolated from the thymus of Jagged1⁺⁺ Lck⁺ and Jagged1⁺⁺ mice (WT, n = 7; KO, n = 6). (B) Statistical analysis of frequencies showing CD4⁺ and CD8⁺ populations isolated from the spleen of Jagged1⁺⁺ Lck⁺ and Jagged1⁺⁺ mice (WT, n = 7; KO, n = 6). (C) Statistical analysis of frequencies showing γδ T cells isolated from the thymus of Jagged1⁺⁺ Lck⁺ and Jagged1⁺⁺ mice (WT, n = 7; KO, n = 6). (D) Statistical analysis of frequencies showing γδ T and αβ T cells isolated from the spleen of Jagged1⁺⁺ Lck⁺ and Jagged1⁺⁺ mice (WT, n = 7; KO, n = 6). Unpaired t test was used for statistical analysis. Each dot represents one mouse. Data represent one out of three independent experiments (mean ± s.d.). ****P < 0.0001, n.s., not significant.
| gene name | WT1 | WT2 | WT3 | KO1 | KO2 | KO3 | log2FoldChange (WT vs KO) | p-value |
|-----------|-----|-----|-----|-----|-----|-----|--------------------------|---------|
| Jagged1   | 1255| 663 | 1305| 326 | 225 | 358 | 1.826228598              | 9.0769E-21 |
| Jagged2   | 293 | 378 | 298 | 255 | 334 | 393 | 0.026889957              | 0.920461617 |
| Notch1    | 1991| 1570| 2222| 2247| 2115| 1996| -0.11629582              | 0.454364945 |
| Notch2    | 1532| 1392| 1240| 985 | 1230| 1055| 0.38310843               | 0.01570432 |
| Notch3    | 15  | 15  | 57  | 43  | 25  | 30  | -0.405671749             | 0.629163724 |
| Notch4    | 16  | 9   | 13  | 6   | 1   | 6   | 1.828551449              | 0.212569814 |
| Hey1      | 753 | 241 | 1165| 183 | 57  | 264 | 2.120194762              | 2.98644E-19 |
| Hes1      | 84  | 43  | 100 | 50  | 24  | 63  | 0.782513394              | 0.163931456 |
| Fabp7     | 419 | 138 | 128 | 145 | 53  | 97  | 1.140357148              | 0.002619491 |
| Gzmb      | 1163| 1091| 2350| 953 | 801 | 1960| 0.364819395              | 0.017419287 |
| Cdkn1a    | 85  | 61  | 156 | 103 | 157 | 344 | -0.676755548             | 0.12057167 |

*SI Appendix, Table S1.* Jagged/Notch signaling-related genes in γδ T cell precursors.
| Gene name | Positive regulator | Negative regulator | log2 Fold Change (WT vs KO) | P-value |
|-----------|--------------------|--------------------|----------------------------|---------|
| Tifa      | Yes                |                    | -1.113396466               | 0.004926847 |
| Pex26     | Yes                |                    | -0.868893167               | 0.002099624 |
| Mcm8      | Yes                |                    | -0.844901334               | 0.001858111 |
| Rad51c    | Yes                |                    | -0.700714852               | 0.013374578 |
| Ercc6l    | Yes                |                    | -0.578009197               | 0.001955554 |
| Bub1      | Yes                |                    | -0.575653436               | 0.000491611 |
| Mcm10     | Yes                |                    | -0.552226674               | 0.000653814 |
| Bub1b     | Yes                |                    | -0.511598679               | 0.001235941 |
| Espl1     | Yes                |                    | -0.507808642               | 0.008622547 |
| Nsl1      | Yes                |                    | -0.500808055               | 0.010352424 |
| Cenpi     | Yes                |                    | -0.488548563               | 0.021651879 |
| Znfx1     | Yes                |                    | -0.476035756               | 0.01502506  |
| Ncapg     | Yes                |                    | -0.457222463               | 0.007912561 |
| Nup188    | Yes                |                    | -0.456208951               | 0.010661018 |
| Mis18bp1  | Yes                |                    | -0.436002266               | 0.006130913 |
| Nup133    | Yes                |                    | -0.431704279               | 0.015454247 |
| Nup214    | Yes                |                    | -0.424318655               | 0.002023248 |
| Ctc1      | Yes                |                    | -0.413488383               | 0.014910158 |
| Exo1      | Yes                |                    | -0.410270085               | 0.015962182 |
| Ncapg2    | Yes                |                    | -0.405121477               | 0.01510686  |
| Uhrf1     | Yes                |                    | -0.396713602               | 0.0023994   |
| Sgo1      | Yes                |                    | -0.394766215               | 0.01943369  |
| Cene2     | Yes                |                    | -0.386580311               | 0.009616668 |
| Pola1     | Yes                |                    | -0.376792697               | 0.025864683 |
| Rad51     | Yes                |                    | -0.374709593               | 0.005079741 |
| Wn        | Yes                |                    | -0.37401211                | 0.017781586 |
| Mast3     | Yes                |                    | -0.373262673               | 0.008984992 |
| Kif2c     | Yes                |                    | -0.364296856               | 0.022055232 |
| Nup210    | Yes                |                    | -0.360622667               | 0.01270739  |
| Cdc45     | Yes                |                    | -0.354314204               | 0.025046459 |
| Mcm2      | Yes                |                    | -0.35402009                | 0.006017997 |
| Ccna2     | Yes                |                    | -0.350371755               | 0.011689978 |
| Cc2d1b    | Yes                |                    | -0.349450059               | 0.016602937 |
| Dyncll2   | Yes                |                    | -0.34355354                | 0.021079118 |
| Stag2     | Yes                |                    | -0.342403179               | 0.011248597 |
| E2f2      | Yes                |                    | -0.341489944               | 0.005968135 |
| Ncapd2    | Yes                |                    | -0.332976232               | 0.022201339 |
| Mre11a    | Yes                |                    | -0.33242003                | 0.020478726 |
| Top2a     | Yes                |                    | -0.324646695               | 0.011689978 |
| Cdc6      | Yes                |                    | -0.323801281               | 0.011730174 |
| Mcm3      | Yes                |                    | -0.322027383               | 0.003660693 |
| Mcm5      | Yes                |                    | -0.317918513               | 0.005390322 |
| Nup37     | Yes                |                    | -0.311713181               | 0.037236513 |
| Cdk6      | Yes                |                    | -0.307467776               | 0.004364171 |
| Igf2bp3   | Yes                |                    | -0.301797564               | 0.043156905 |
| Tubgcp5   | Yes                |                    | -0.296867224               | 0.048035984 |
| Rrm2      | Yes                |                    | -0.277254826               | 0.016014386 |
| Ccnb2     | Yes                |                    | -0.26996143                | 0.015478581 |
| Smc2      | Yes                |                    | -0.268902447               | 0.016978853 |
| Ncaph     | Yes                |                    | -0.267732075               | 0.032293081 |
| Xpol      | Yes                |                    | -0.266886446               | 0.038141044 |
| Spc24     | Yes                |                    | -0.264120544               | 0.03626624  |
| Gene | Yes/No | Mean | Standard Deviation |
|------|--------|------|--------------------|
| Haus4 | Yes | -0.262322168 | 0.041868416 |
| Cdk1  | Yes | -0.247148451 | 0.044819053 |
| Fen1  | Yes | -0.235056559 | 0.049330831 |
| Smcl1a| Yes | -0.227532356 | 0.034521925 |
| Mcrn7 | Yes | -0.215038027 | 0.034453273 |

*SI Appendix, Table S2.* Cell cycle-related genes in γδ T cell precursors based on RNA-seq data.
| gene name | WT1 | WT2 | WT3 | KO1 | KO2 | KO3 | log2FoldChange (WT vs KO) | p-value |
|-----------|-----|-----|-----|-----|-----|-----|---------------------------|---------|
| Jagged1   | 203 | 216 | 249 | 212 | 145 | 215 | 0.358233114               | 0.194812626 |
| Jagged2   | 331 | 561 | 460 | 418 | 568 | 411 | 0.05357539                | 0.792988749 |
| Notch1    | 2782| 2838| 2825| 2901| 3301| 3138| -0.025791827              | 0.829597109 |
| Notch2    | 691 | 1050| 907 | 871 | 893 | 1080| 0.000834651               | 0.99570365  |
| Notch3    | 148 | 118 | 133 | 160 | 217 | 245 | -0.508482129              | 0.117469966 |
| Notch4    | 24  | 11  | 21  | 15  | 19  | 11  | 0.409233495               | 0.656321428 |
| Hey1      | 104 | 100 | 44  | 178 | 50  | 31  | 0.343379018               | 0.461282649 |
| Hes1      | 669 | 279 | 335 | 430 | 269 | 359 | 0.31951817                | 0.172967334 |
| Fabp7     | 64  | 29  | 22  | 25  | 20  | 33  | 0.561263123               | 0.419277938 |
| Gzmb      | 404 | 283 | 248 | 557 | 240 | 291 | -0.038069091              | 0.867291985 |
| Cdkn1a    | 56  | 37  | 64  | 64  | 25  | 13  | 0.95501405                | 0.139897043 |

*SI Appendix, Table S3.* Jagged/Notch signaling-related genes in γδ T mature cells.
| Antibodies or reagents | Supplier       | Conjugated   | Catalog number | Clone number |
|------------------------|---------------|--------------|----------------|--------------|
| Annexin V              | Biolegend     | APC          | 640941         |              |
| BrdU                   | Biolegend     | PERCP-CY5.5  | 364110         | 3D4          |
| CD3                    | Biolegend     | APC-CY7      | 100222         | 17A2         |
| CD3                    | Biolegend     | APC          | 100236         | 17A2         |
| CD3                    | Biolegend     | PERCP-CY5.5  | 100218         | 17A2         |
| CD4                    | Biolegend     | FITC         | 100204         | 17A2         |
| CD4                    | Biolegend     | BV605        | 100451         | GK1.5        |
| CD4                    | Biolegend     | BV711        | 100447         | GK1.5        |
| CD8α                   | Biolegend     | BV605        | 100744         | 53-6.7       |
| CD8α                   | Biolegend     | BV711        | 100759         | 53-6.7       |
| CD8β                   | Biolegend     | APC          | 100712         | 53-6.7       |
| CD8β                   | eBioscience   | FITC         | 50-112-8937    | H35-17.2     |
| CD24                   | Biolegend     | AF700        | 101836         | M1/69        |
| CD25                   | Biolegend     | PE           | 102008         | PC61         |
| CD25                   | Biolegend     | PERCP-CY5.5  | 101912         | 3C7          |
| CD44                   | Biolegend     | APC-CY7      | 103028         | IM7          |
| CD44                   | Biolegend     | APC          | 103012         | IM7          |
| CD44                   | Biolegend     | BV421        | 103040         | IM7          |
| CD45.2                 | Biolegend     | BV510        | 109838         | 104          |
| CD45.2                 | Biolegend     | FITC         | 109806         | 104          |
| CD62L                  | Biolegend     | BV421        | 104435         | MEL-14       |
| CD73                   | Biolegend     | PE-CY7       | 127224         | TV/11.8      |
| CD339 (Jagged1)        | Biolegend     | PE           | 130907         | HMK1-29      |
| NK1.1                  | Biolegend     | BV421        | 108741         | PK136        |
| NK1.1                  | Biolegend     | PERCP-CY5.5  | 108728         | PK136        |
| Notch2                 | Biolegend     | APC          | 130713         | HMM2-35      |
| TCRβ                   | Biolegend     | APC          | 109212         | H57-597      |
| TCRβ                   | Biolegend     | FITC         | 109206         | H57-597      |
| TCRγ/δ                 | Biolegend     | PE-CY7       | 118124         | GL3          |
| TCRγ/δ                 | Biolegend     | FITC         | 118106         | GL3          |
| TCRγ1.1                | Biolegend     | APC          | 141108         | 2.11         |
| TCRγ2                  | Biolegend     | PE           | 137706         | UC3-10A6     |
| IL-17A                 | Biolegend     | BV421        | 512322         | BL168        |
| IL-17A                 | Biolegend     | PE           | 506904         | TC11-18H10.1 |
| IL-4                   | Biolegend     | APC          | 504106         | 11B11        |
| IFN-γ                  | Biolegend     | BV421        | 505830         | XMG1.2       |
| IFN-γ                  | Biolegend     | PE           | 505808         | XMG1.2       |
| Ki67                   | BD Biosciences| BV510        | BDB562899      | B56          |
| Ki67                   | Biolegend     | APC          | 652406         | 16A8         |
| 7AAD                   | Thermo Fisher  | PERCP-CY5.5  | A1310          |              |
| BD Cytoperm™ Permeabilization Buffer Plus | BD Biosciences | | 561651 | |
| BD Cytofix/Cytoperm Fixation/Permeabilization Concentrate and Diluent | eBioscience | | 00-5521-00 | |

*SI Appendix, Table S4.* Antibodies and reagents.
Supporting Information Materials and Methods

In vivo S. Typhimurium infection. Salmonella enterica subsp. enterica serovar Typhimurium (SL1344 strain) was provided by J. Galan and stocked in our lab as previously described (1). Routinely, 8 to 10 weeks old mice were fasted for 4 hours followed by gavage of streptomycin (20 mg), at day -1. At day 0 of infection, mice were fasted again for 4 hours and infected with 1 × 10^3 CFUs of S. Typhimurium per mouse. Bacteria were propagated overnight in LB containing streptomycin (100 μg/ml) before infection, and subcultured in high salt LB medium (0.3M NaCl) with no antibiotics. Bacterial CFU was calculated by using spectrophotometry with OD600nm wavelength. To calculate fecal CFU, feces were collected at day 4 post infection and resuspended in PBS at the ratio of 50mg feces per ml PBS. After being vortexed for 30 min, bacteria containing supernatants were clarified by centrifugation at 50g for 10 min. The supernatants were serially diluted and plated on LB streptomycin plates (100 μg/ml) with 10μl samples each dot. Similarly, caecum, spleen and liver tissues were harvested and dissociated with gentleMacs C Tubes (Miltenyi Biotec, #130-096-334) as previously described (1). Organs CFU counts were calculated using similar methodology as above. All CFU counts were preformed blinded by two experimenters separately. All animal experimentats were performed in compliance with Yale Institutional Animal Care and Use Committee protocols.

Colon lymphocytes isolation. Colon tissues was harvested as described and kept in cold RPMI medium until next procedure, when they were turned inside out and incubated in 25ml dissociation medium (RPMI with 1mM DTT, 2mM EDTA and 2% FBS). The tissues were kept stirring at the speed of 500rpm in 37°C incubation boxes for 30 minutes. The supernatant which contains the intraepithelial lymphocytes (IEL) was centrifuged and washed with FACS buffer (DPBS with 2% FBS). The remaining colon tissues were cut into small pieces and incubated with 20ml digestion medium (RPMI with 0.5mg/ml Dispase, 1mg/ml Type II Collagenase and 2% FBS), For the digestion procedure, tissues were treated for 45-60 minutes at 37°C, with 600rpm of stirring speed. When digestion was over, the samples were filtered through 70μm cell strainer, and rinsed with additional 25ml of FACS buffer, followed by centrifuging at 500g for 10 minutes. The cell pellet which contains the lamina propria lymphocytes (LPL) was collected and washed with FACS buffer. Each collected IEL and LPL samples were purified using 40% percoll, and then were strained to remove any undigested material and washed in FACS buffer before processing for flow cytometry analysis.

Flow cytometry. For surface staining, single-cell suspensions were prepared as described and stained for surface markers for 15-30 min at 4 °C. For intracellular cytokine staining, the cells were re-stimulated for 3 h at 37°C with phorbol 12-myristate 13-acetate (PMA) (Sigma, 50 ng/ml) and ionomycin (Sigma, 1 μg/ml) in the presence of Monensin (Biolegend). After stimulation, cells were washed and stained as the manufacturer described (BD, #554714). For staining of nuclear factors, cells were fixed and stained according to the manufacturer’s instructions (eBioscience, #00-5523-00). For BrdU experiment, mice were retro-orbital injected with BrdU at a dose of 100 μg per gram of body weight 2 hours before euthanasia. Then thymocytes were collected and stained as described above. Cells were all re-suspended in PBS, 0.5% FBS, 5 mM EDTA and acquired with an LSRII cytometer (BD Bioscience). Data
were analyzed by using FlowJo software (version 9.0 or higher, BD Bioscience). The list of antibodies and reagents used in flow cytometry is shown in SI Appendix, Table S4.

**RNA isolation and qPCR.** TRizol reagent (Invitrogen) was added to total thymic lymphocytes and then processed following the manufacturer’s instructions. RNA was further purified using the RNase-Free DNase Set (QIAGEN, #79256). The RNA isolation of γδ T cells or progenitors was performed with RNeasy Micro Kit (QIAGEN, #74034) following the manufacturer’s protocol. Maxima H Minus Reverse Transcriptase Kit (ThermoFisher Scientific, #EP0753) was used for cDNA synthesis. Sigma KiCqStart predesigned SYBR green primers, gene-specific 6FAM-MGB probes (ThermoFisher Scientific) and iTaq Universal SYBR Green Supermix or FAM Supermix (Biorad) were used for real time PCR, and β-actin mRNA level was used as internal control to calculated mRNA relative abundance. Primer sequences used for qPCR are as follow: mouse *Alkbh5* (forward, 5’-CGCGGTACGACTACC-3’; reverse, 5’-ATGGGCTTTGAACTTGTTG-3’), mouse *β-actin* (forward, 5’-AGTGTGACGTTGACATCCGT-3’; reverse, 5’-GCAGCTCAGTAAGCTCCGC-3’), mouse *Myc-peak* (forward, 5’-GCTTGGAAACTCTGGTGCAT-3’; reverse, 5’-AATTCCAGCGCATGTTCT-3’). Mouse *Jagged1, Notch1, Notch2, Hey1, Hes1, Cdkn1a* and *β-actin* specific probes (ThermoFisher Scientific, #4453320).

**Western blot.** Total protein of thymic lymphocytes was extracted with RIPA lysis buffer supplemented with protease inhibitors (ThermoFisher Scientific). Antibodies against ALKBH5 (Proteintech, #16837-1-AP) and β-actin (Cell Signaling Technology, #4970) were used at a 1:1,000 dilution in 5% no-fat milk buffer at 4 °C overnight. Membranes were washed with 0.1% PBST buffer and then incubated in HRP-conjugated secondary antibody (Cell Signaling Technology, #7074) at room temperature for 1 hour. After extensive wash, membranes were incubated in TLC chemiluminescence reagent (Biorad) and exposed to X-ray film.

**m⁶A dot blot.** m⁶A dot blot was conducted as previously described (2). Briefly, total RNA extracted from total thymic γδ T cells, were purified with the Dynabeads mRNA Purification Kit (Invitrogen, #61006) according to the manufacturer’s instructions. Then, purified mRNA was further mixed with gly sample loading dye (Invitrogen, #AM8551) and denatured at 65 °C for 20 min, and put on ice immediately. Denatured samples were dropped onto the Hybond-N+ membrane, dried naturally and performed UV crosslinking for 2 minutes. 0.1% PBST wash buffer was used to wash the membranes, which were then blocked with 5% non-fat milk for 1 hour. Later, the membranes were probed with anti-m⁶A antibody (Synaptic Systems, #202003) at a dilution of 1:1000 in 4 °C for overnight with gentle shaking. 0.1% PBST buffer washed the membranes three times, 10 min each time. After that, an HRP-conjugated secondary antibody (Cell Signaling Technology, #7074) was added to the membranes and incubated at room temperature for 1 hour. After extensive wash, the signal was detected by enhanced chemiluminescence with pico ECL using Chemidoc MP (Biorad).

**RNA-seq library preparation and data processing.** Thymic mature γδ T cells (CD45.2⁺CD3⁺TCRγδ⁺) and γδ T progenitors (CD45.2⁺CD4⁻CD8⁻TCRγδ⁺CD73⁺) were sorted by FACS Aria II (BD Bioscience). Total RNAs were isolated by RNeasy Micro Kit (QIAGEN,
(YCGA) using SMARTer® Stranded Total RNA-Seq Kit-Pico Input Mammalian (Clontech). The libraries were sequenced on Illumina HiSeq X 4000 platform (paired-end 150-bp read length) by Geneseq Technology Inc. Each sample obtained 50 million reads. STAR was used to align the raw sequencing reads to mouse genome mm10 with default parameters after trimming adapter sequences with cutadapt (3, 4). Subsequently, we counted reads in features with hts-sequ-count and used DESeq2 for identifying differentially expressed genes between the WT and KO samples with the same number of biological replicates (5). Visualizations using volcano-plots and heatmaps were produced with R. Gene Ontology (GO) enrichment analysis of biological process was performed by Reactome signaling pathway analysis based on differentially expressed genes.

m6A-RIP-qPCR. m6A-RIP-qPCR analysis was performed as described previously (2). Briefly, total thymic γδ T cells were isolated using mouse TCRγδ+ T Cell Isolation Kit (Miltenyi Biotec, #130-092-125) and processed according to the manufacturer’s protocol. The purified poly(A)+ mRNA was incubated with anti-m6A antibody (Synaptic System, #202003,) or rabbit IgG in RIP wash buffer (150 mM NaCl, 10 mM Tris, 0.1 % NP40, PH 7.4, supplemented with RNase inhibitor) for 2 hours at 4 °C. Then, Protein A beads (ThermoFisher Scientific, #21348) were added and incubated in 4 °C for 2 hours. After extensive wash with RIP buffer, immunoprecipitated beads-m6A antibody-mRNA complex was competitively eluted by m6A nucleotide by using TRizol reagent (Invitrogen), and then purified by 75 % ethanol precipitation. Maxima H Minus Reverse Transcriptase Kit (ThermoFisher Scientific, #EP0753) was used for cDNA synthesis. Sigma KiCqStart predesigned SYBR green primers, gene-specific 6FAM-MGB probes (ThermoFisher Scientific) and iTaq Universal SYBR Green Supermix or FAM Supermix (Biorad) were used for real time PCR. β-actin as m6A negative control, Myc peak as m6A positive control (6).

RNA decay analysis. RNA decay analysis was conducted as previously described (7). Briefly, total thymic γδ T cells were isolated using mouse TCRγδ+ T Cell Isolation Kit (Miltenyi Biotec, #130-092-125) and processed following the manufacturer’s instruction. Then, cells were plated on 96-well plates with 5 × 10^4 cells per well. After treatment with actinomycin D (5 μg/ml) (Sigma-Aldrich, #A1410) for 0, 2 hours and 4 hours, cells were collected and subjected to RNA extraction. Total RNAs isolation and qPCR conducted for mRNA levels as described above.

LC-MC analysis. The principal pipeline for quantifying m6A RNA modification by LC-MS was described as before (8). Basically, mRNAs were isolated from the thymocytes of Alkbh5f/f Lck-Cre mice and Alkbh5f/f mice by using mRNA isolation kit (NEB, S1550S). Later mRNAs were digested with first nuclease P1 (NEB, M0660S) and then Antarctic Phosphatase (NEB, M0289S) to generate adenosine and N6-adenosine molecules. The quantification of adenosine and N6-adenosine was achieved utilizing high-resolution ESI-MS (HR-ESI-MS) via an Agilent iFunnel 6550 (Santa Clara, CA) quadrupole time-of-flight (QTOF) MS instrument fitted with an electrospray ionization (ESI) source (positive mode) coupled to an Agilent 1290 Infinity high performance liquid chromatography (HPLC) system. A Phenomenex Luna C18(2) (100 Å, 5 m,
4.6 × 150 mm) (Phenomenex, CA, USA) column was employed for the quantification with a gradient program starting from 2% to 10% acetonitrile over 15 min (retention times: adenosine at 7.6 min and N6-adenosine 11.2 min). Each concentration of the nucleosides was deduced by a standard curve generated by injection of a series of concentrations of authentic standards (0.1, 0.01, 0.001 and 0.0001 µg/mL) followed by integration of each ion count from samples. Data presented in Fig. 5B is mass concentration of N6-adenosine molecules normalized to 1µg/ml adenosine in each sample.

**Statistical analysis.** Statistical analysis was calculated in GraphPad Prism 8 series software. Paired or unpaired Student's t-test and two-tailed Mann–Whitney U test were used for measurement data of two groups analysis. All general statistical analysis was calculated with a confidence interval of 95%. P values ≤ 0.05 were considered as statistically significant. Survival curves were compared by the log-rank test using GraphPad Prism 8. Data are represented as mean ± s.e.m. or mean ± s.d. as indicated in the figures.

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