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Supplementary Materials for

Sphingolipid biosynthesis is essential for metabolic rewiring during T_H17 cell differentiation

Thiruvaimozhi Abimannan *et al.*

Corresponding author: Jairaj K. Acharya, acharyaj@mail.nih.gov; Usha Acharya, acharyaur@nih.gov

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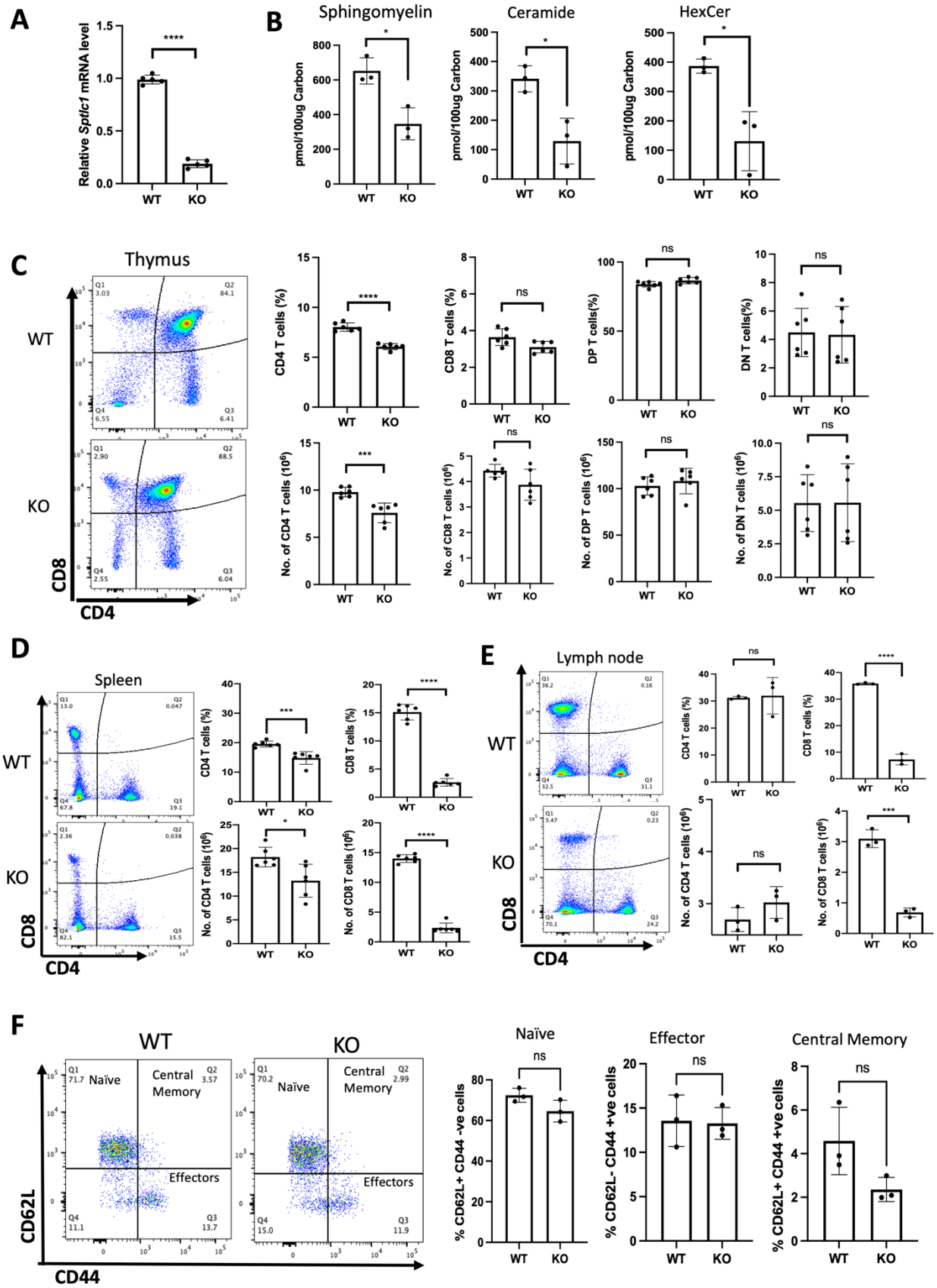
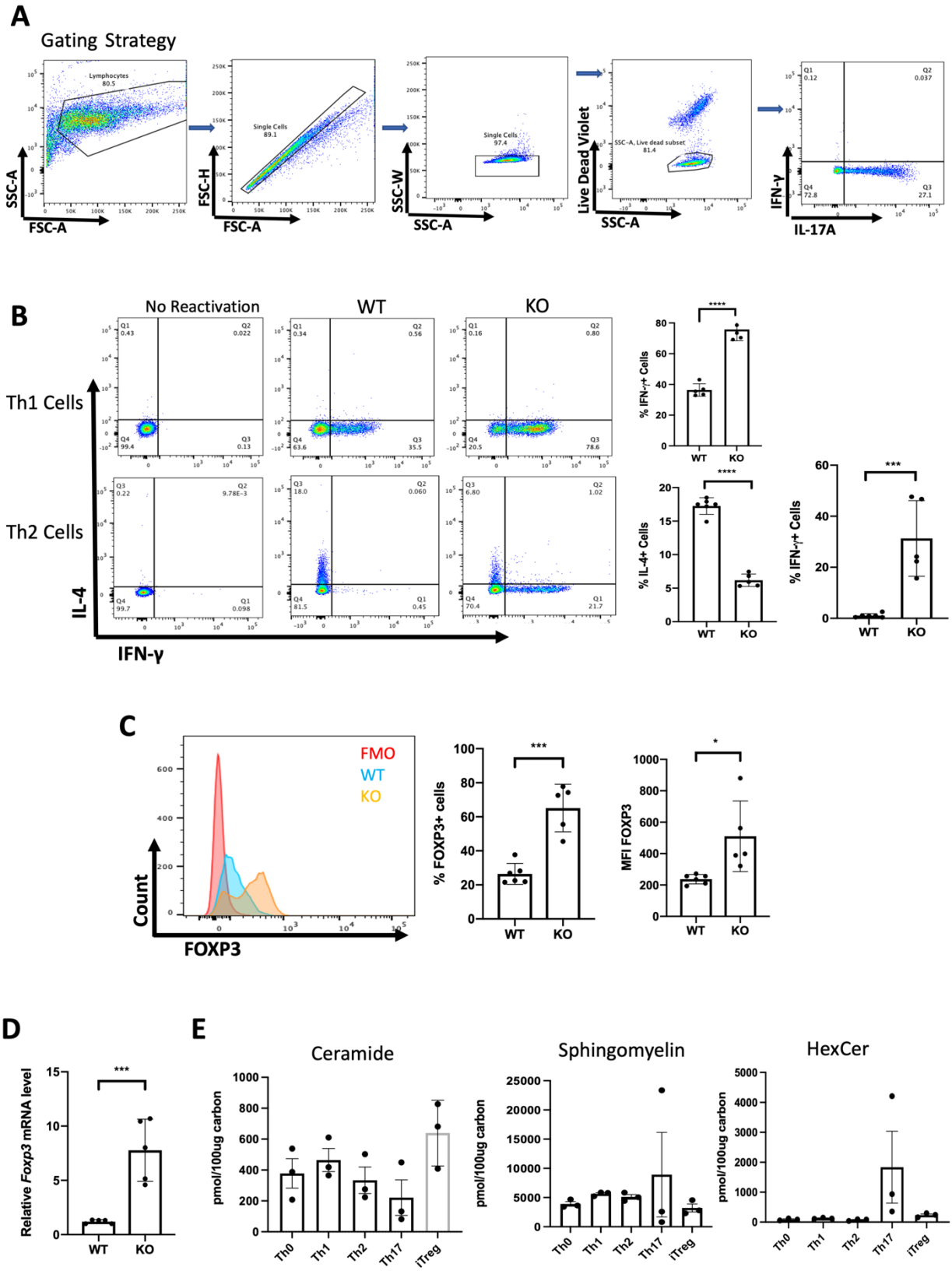


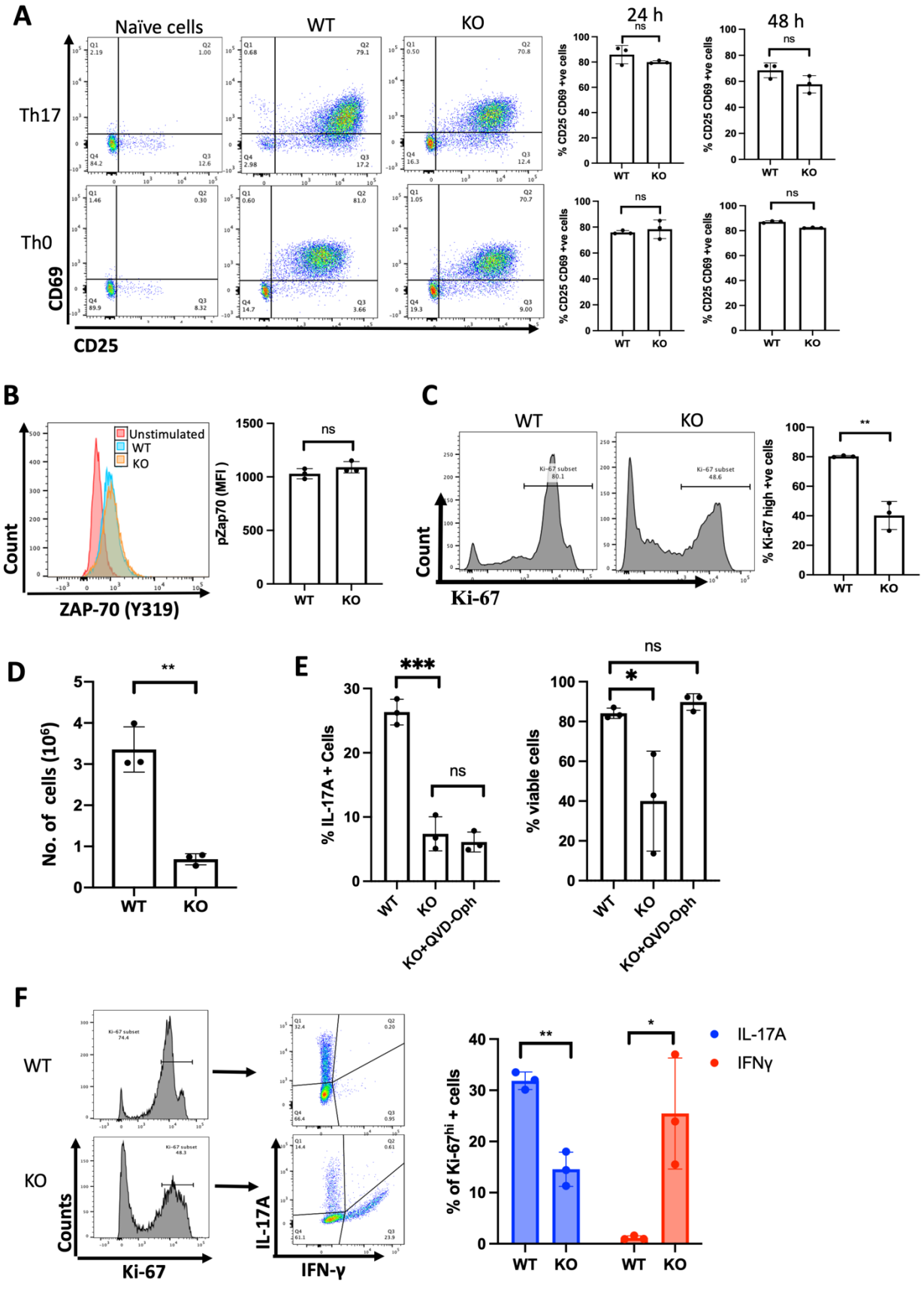
Supplementary Figure 1, Abimannan et al., 2024

Fig. S1. SPTLC1 is essential for Th17 differentiation *in vitro*. (A) WT and KO T cells were differentiated into Th17 subsets and *Sptlc1* mRNA quantified by qPCR. n=5 biologically independent samples. **(B)** WT and KO naïve T cells were analyzed for indicated sphingolipids species by mass spectrometry. Sphingolipids levels expressed in picomoles (pmol)/100 micrograms of carbon. n=3 biologically independent samples. **(C)** Thymocytes from WT and KO mice stained for CD4 and CD8 and analyzed by flow cytometry. Left panel is a representative flow figure and right upper and right bottom panel shows the frequency and absolute number $CD4^+$, $CD8^+$ and $CD4^+CD8^+$ double positive cells respectively. n=6 biologically independent samples. **(D)** Splenocytes from WT and KO mice stained for CD4 and CD8 and analyzed by flow cytometry. Left panel is a representative flow figure and right upper and right bottom panel shows the frequency and absolute number $CD4^+$ and $CD8^+$ positive cells respectively. n=6 biologically independent samples. **(E)** Lymph node cells from WT and KO mice stained for CD4 and CD8 and analyzed by flow cytometry. Left panel is a representative flow figure and right panel shows the cumulative data of the same. Right upper and right bottom panel shows the frequency and absolute number $CD4^+$ and $CD8^+$ positive cells respectively. n=3 biologically independent samples. **(F)** Splenocytes from WT and KO mice stained for CD44 and CD62L and analyzed by flow cytometry. Left panel is a representative flow figure and right panel shows the cumulative data for naïve ($CD62L^{high}$ $CD44^{low}$), effector ($CD62L^{low}$ $CD44^{high}$) and central memory ($CD62L^{high}$ $CD44^{high}$) cells. n=3 biologically independent samples. Each dot represents an individual mouse. All data presented as mean ± SEM: *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001 and ns, not significant.



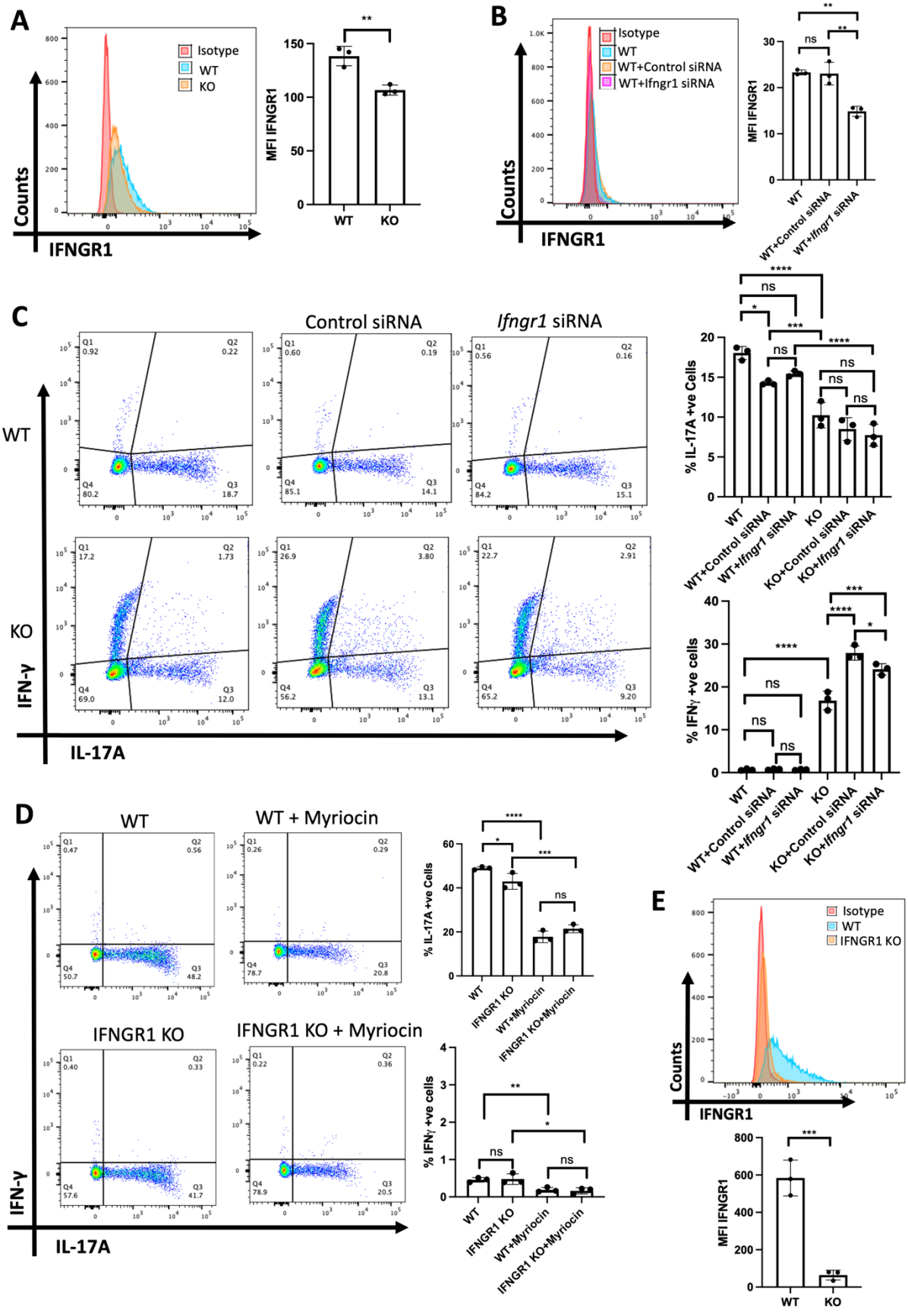
Supplementary Figure 2, Abimannan et al., 2024

Fig. S2. SPTLC1 is required for Th17 differentiation *in vitro*. (A) Gating strategy for analyzing intracellular cytokines. **(B)** WT and KO naïve T cells were differentiated into Th1 or Th2 cells for 4 days and intracellular cytokines IFN-γ^+ (for Th1) and IL-4 (for Th2) were scored by flow cytometry. Left panel is a representative flow figure and right panel shows the cumulative data for the same. n=4-5 biologically independent samples. **(C)** WT and KO naïve T cells were differentiated into iTreg cells for 4 days and analyzed for expression of FOXP3 by flow cytometry. Left panel is a representative figure and right panel shows the cumulative data. n=4-5 biologically independent samples, representative of 3 independent experiments. **(D)** WT and KO naïve T cells were differentiated into iTreg cells for 3 days and *Foxp3* mRNA quantified by qPCR. n=5 biologically independent samples. **(E)** WT naïve T cells were differentiated into different subsets and analyzed for sphingolipids species ceramide, sphingomyelin and HexCer by mass spectrometry. Sphingolipids levels Sphingolipids levels expressed in picomoles (pmol)/100 micrograms of carbon. n=3 biologically independent samples. Each dot represents an individual mouse. All data presented as mean ± SEM: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ and ns, not significant.



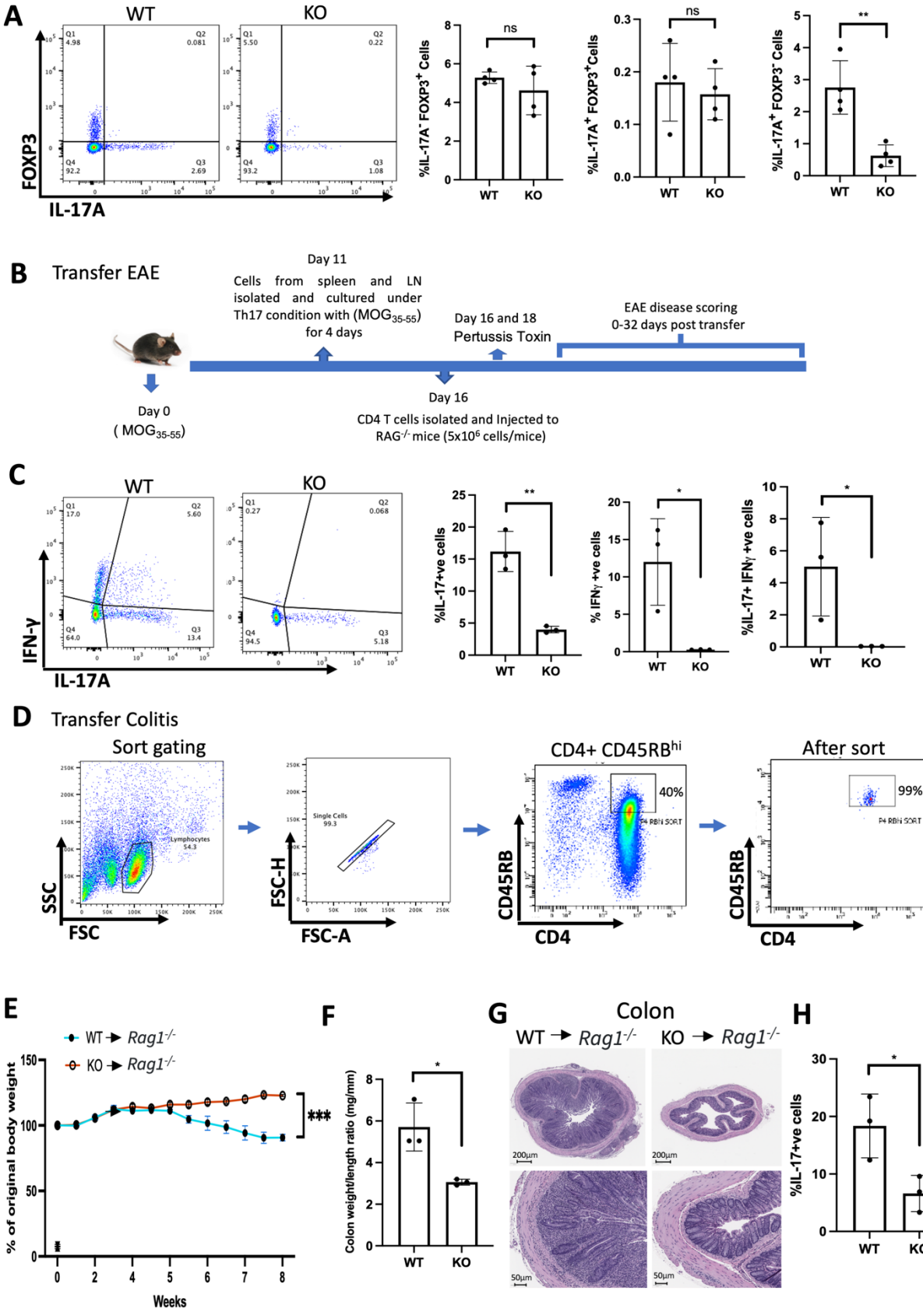
Supplementary Figure 3, Abimannan et al., 2024

Fig. S3. SPTLC1 is essential for Th17 differentiation *in vitro*. (A) Naïve $CD4^+$ T cells from WT and KO mice were activated under indicated subset differentiating conditions for 24 and 48 hours and scored for surface expression of CD69 and CD25 by flow cytometry. Left panel is a representative flow figure and right panel shows the cumulative data of the same. n=3 biologically independent samples. **(B)** WT and KO naïve T cells were crosslinked with anti-CD3 for 15 min and scored for phospho-ZAP-70 by flow cytometry. Left panel is a representative flow figure and right panel shows the cumulative data of phospho-ZAP-70 MFI for the same. n=3 biologically independent samples. **(C)** WT and KO naïve cells were differentiated under Th17 condition for 4 days and scored for frequency of Ki-67^+ cells by flow cytometry. Left panel is a representative flow figure and right panel shows the cumulative percent Ki-67^{hi} positive cells. n=3 biologically independent samples. **(D)** WT and KO naïve cells were differentiated under Th17 condition for 4 days and live cells were counted by trypan blue staining. n=3 biologically independent samples. **(E)** WT and KO naïve cells were differentiated under Th17 condition with or without QVD-Oph for 4 days and analyzed for frequency of IL-$17A^+$ cells (left panel) %viable cells (right panel) by flow cytometry. n=3 biologically independent samples. **(F)** Naïve $CD4^+$ T cells from WT or KO mice were differentiated into Th17 cells for 4 days and frequency of IL-$17A^+$ and IFN-γ^+ cells were analyzed in Ki-67^{hi} positive cells. Left panel is representative flow figure and right panel shows the cumulative data of the same. n=3 biologically independent samples. Each dot represents an individual mouse. All data presented as mean ± SEM: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ and ns, not significant.



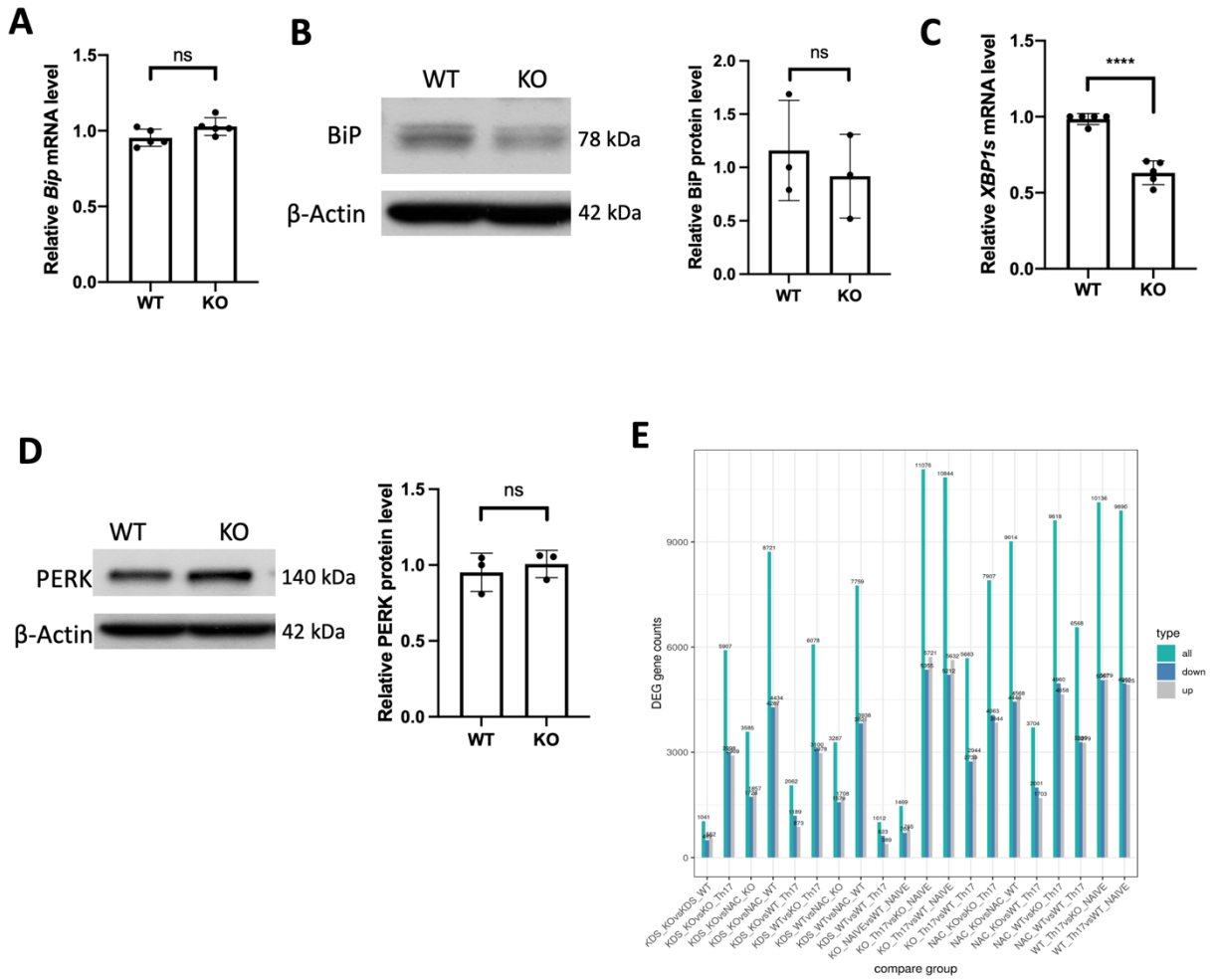
Supplementary Figure 4, Abimannan et al., 2024

Fig. S4. SPTLC1 is required for Th17 differentiation *in vitro*. (A) WT and KO naïve T cells were differentiated into Th17 cells for 4 days and scored for surface expressed IFNGR1 by flow cytometry. Left panel is a representative flow figure and right panel shows the cumulative data of the same. n=3 biologically independent samples. **(B)** Naïve WT $CD4^+$T cells were differentiated into Th17 cells in the presence of control or IFNGR1 siRNA for 4 days and scored for surface expressed IFNGR1 by flow cytometry. Left panel is a representative flow figure and right panel shows the cumulative data of the same. n=3 biologically independent samples. **(C)** WT and KO naïve $CD4^+$ T cells were differentiated into Th17 cells for 5 days with the addition of control siRNA or IFNGR1 siRNA after 24 hours of differentiation. 5 days later cells were stimulated with PMA/Ion and scored for indicated intercellular cytokines. Left panel is a representative flow figure and right panel shows the cumulative data of the same. n=3 biologically independent samples. **(D)** WT and KO naïve $CD4^+$ T cells were differentiated into Th17 cells in the presence or absence of Myriocin for 4 days and scored for intracellular cytokines by flow cytometry. Left panel is a representative flow figure and right panel shows the cumulative data of the same. n=3 biologically independent samples. **(E)** WT and IFNGR1 KO naïve T cells were differentiated into Th1 cells for 4 days and scored for surface expressed IFNGR1 by flow cytometry. Top panel is a representative flow figure and bottom panel shows the cumulative data (WT or KO MFI – Isotype MFI) of the same. n=3 biologically independent samples. Each dot represents an individual mouse. All data presented as mean ± SEM: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ and ns, not significant.



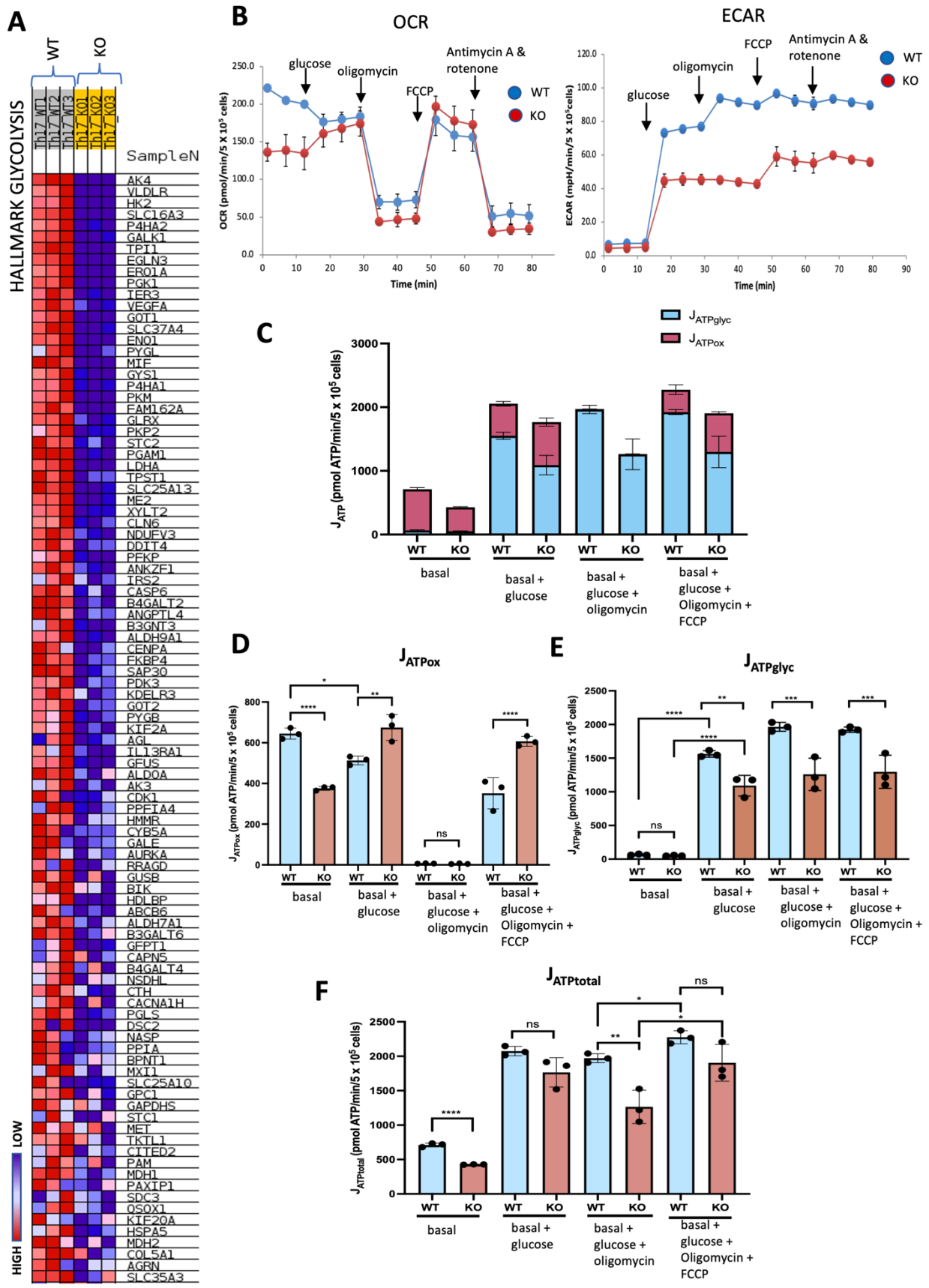
Supplementary Figure 5, Abimannan et al., 2024

Fig. S5. SPTLC1 is required for Th17 differentiation *in vivo*. (A) WT and KO mice were immunized with MOG_{35-45} peptide with pertussis toxin. On day 8 (disease pre-onset stage) splenocytes were analyzed for indicated transcription factor and cytokines**.** Left panel is a representative flow figure and right panel shows the cumulative data of the same. n=3 biologically independent samples. **(B)** Schematic of experimental design for T cell transfer EAE. **(C)** WT and KO mice were immunized with MOG_{35-45} peptide and splenocytes were isolated after 11 days and cultured under Th17 cytokine milieu as described in methods. 4 days later $CD4^+$ T cells were isolated and stimulated with PMA/Ion and scored for indicated intercellular cytokines. WT (n=3 biological replicates) KO (n=6; 2 mice per biological replicates). **(D)** Gating strategy for sorting $CD4^+$ $CD45RB^{hi}$ cells by FACSAria. $CD4^+CD45RB^{hi}$ cells were sorted from WT and KO mice and equal number of viable cells were adoptively transferred to $Rag^{-/-}$ host mice. **(E)** Body weight changes of $Rag^{-/-}$ recipients of $CD4^+CD45RB^{hi}$ naïve cells from WT and KO mice at indicated time points. n=3 recipient mice per group. **(F)** Colon length to weight/length ratio of $Rag^{-/-}$ recipients of $CD4^+$ $CD45RB^{hi}$ naïve $CD4^+$ T cells from WT and KO mice, 8 weeks after transfer. n=3 recipient mice. **(G)** Representative H&E-stained colon sections of $Rag^{-/-}$ recipients of naïve CD4 T cells from WT and KO mice, 8 weeks after transfer. $Rag^{-/-}$ recipients of naïve CD4 T cells from WT showing severe colitis with hyperplastic crypt epithelium and inflammatory cell infiltration. Upper panel, scale bar, 200µm; lower panel, scale bar 50µm. n=3 recipient mice per group. **(H)** The frequency of $IL\text{-}17A^+$ cells in lamina propria (LP) lymphocytes isolated from small intestine and colon of $Rag^{-/-}$ recipients of $CD4^+$ $CD45RB^{hi}$ naïve $CD4^+$ T cells from WT and KO mice, 8 weeks after transfer. n=3 recipient mice per group. Each dot represents an individual mouse. All data presented as mean ± SEM: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ and ns, not significant.



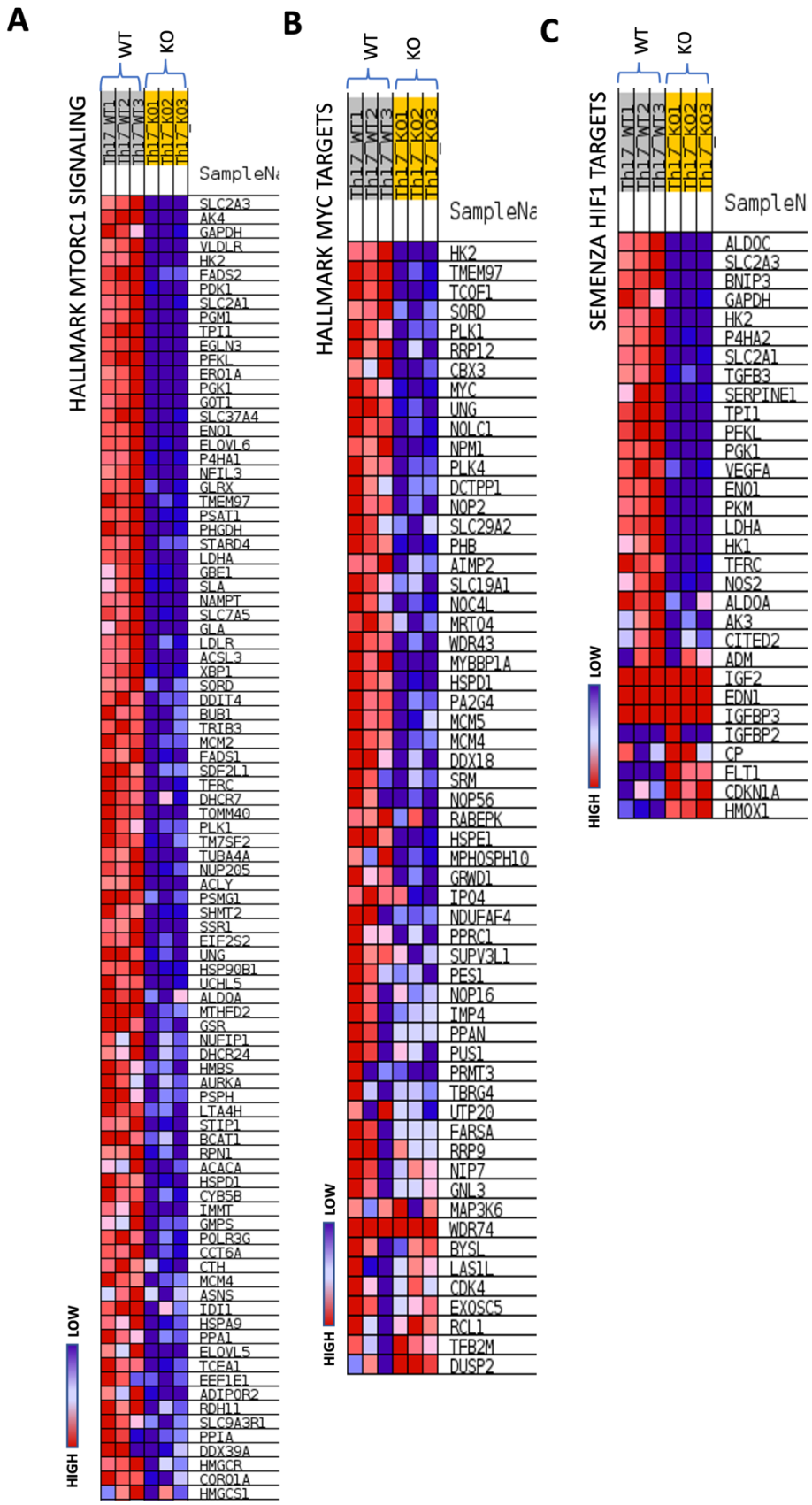
Supplementary Figure 6, Abimannan et al., 2024

Fig. S6. SPTLC1 is required for metabolic reprograming in Th17 differentiation. (A-D) ER stress markers analyzed in WT and KO naïve $CD4^+$ T cells differentiated into Th17 cells for 4 days. **(A)** *bip* mRNA quantified by qPCR. n=5 biologically independent samples **(B)** Immunoblot of Bip. Left panel shows representative western blot and the right panel is the quantitative data for the same. n=3 biologically independent samples **(C)** *XBP1s* mRNA quantified by qPCR. n=5 biologically independent samples **(D)** Immunoblot of PERK. Left panel shows representative western blot and the right panel is the quantitative data for the same. n=3 biologically independent samples. Each dot represents an individual mouse. All data presented as mean ± SEM: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ and ns, not significant. **(E)** Differentially expressed gene (DEG) counts in indicated conditions. **(F)** Pathway enrichment analysis of DEG in WT Th17 cells compared to KO Th17 cells.



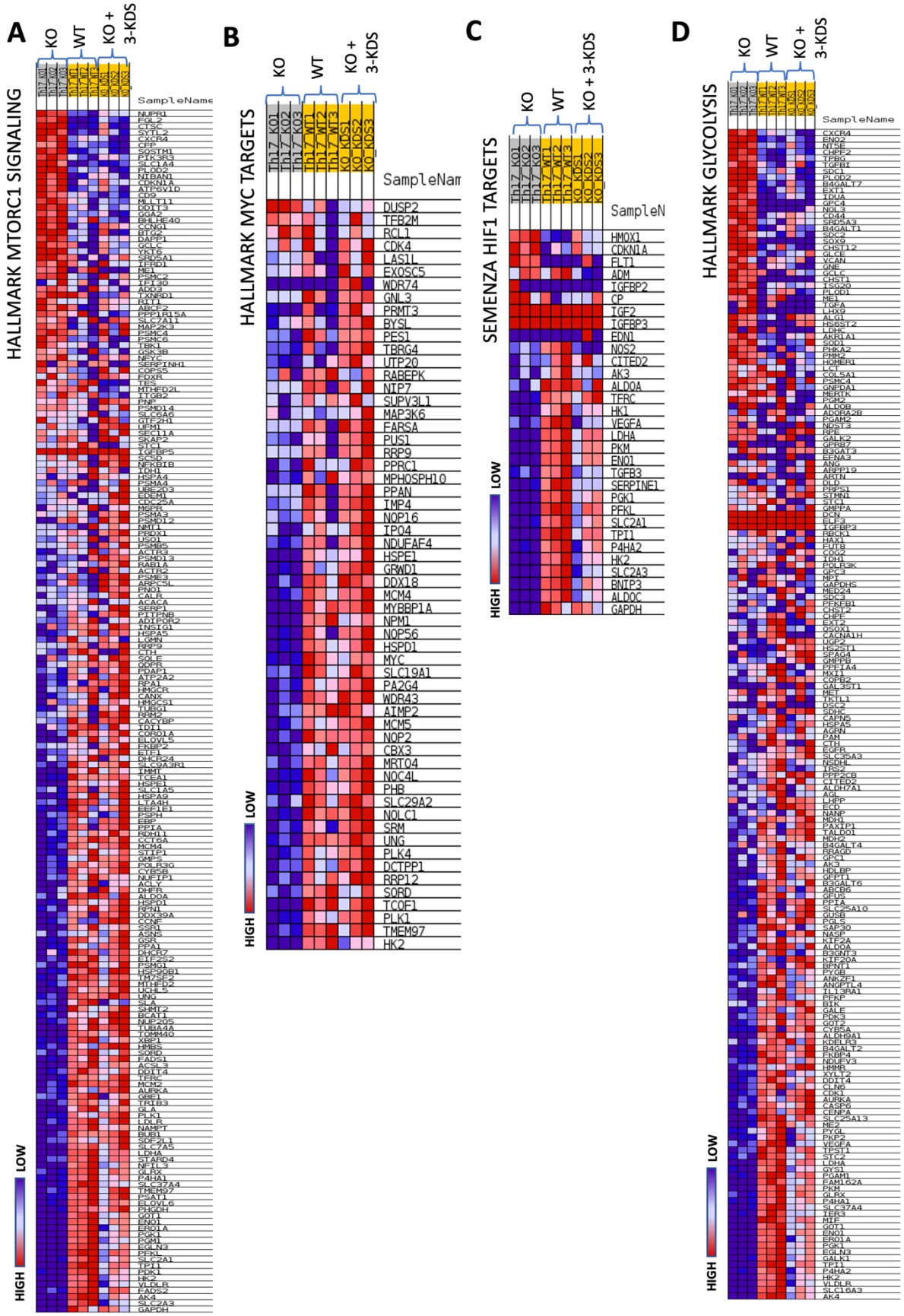
Supplementary Figure 7, Abimannan et al., 2024

Fig. S7. SPTLC1 is required for metabolic reprograming in Th17 differentiation. (A) Heat map displaying the genes of gene set 'HALLMARK_GLYCOLYSIS' in WT and KO Th17 cells. **(B)** Raw traces of OCR and ECAR by Th17 cells. The measurements were taken under basal condition (without exogenous substrate) followed by sequential addition of glucose, oligomycin, carbonyl cyanide-p-trifluoromethoxyphenylhydrazone (FCCP) and rotenone plus antimycin A. Representative figure of one WT and KO mice of n=3 biologically independent samples. $J_{ATPglyc}$ and J_{ATPox} by using Brad and Mookerjee methods and presented in C-F. **(C)** Stacked graph showing $J_{ATPglyc}$ and J_{ATPox} for each injection in WT and KO Th17 cells. n=3 biologically independent samples. **(D)** J_{ATPox} for each injection in WT and KO Th17 cells. **(E)** $J_{ATPglyc}$ for each injection in WT and KO Th17 cells. **(F)** $J_{ATPtotal}$ for each injection in WT and KO Th17 cells. n=3 biologically independent samples. Each dot represents an individual mouse. All data presented as mean ± SEM: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ and ns, not significant.



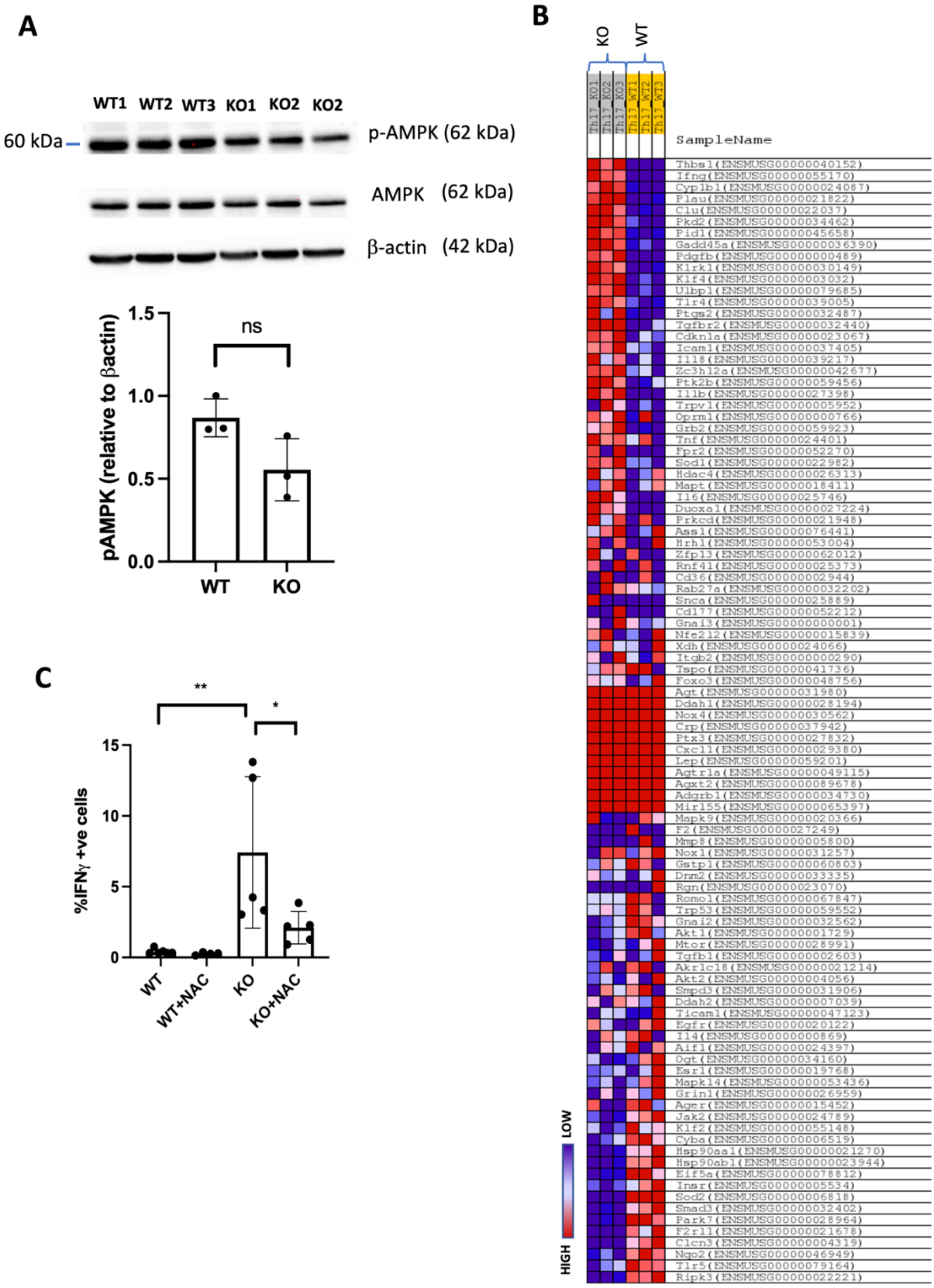
Supplementary Figure 8, Abimannan et al., 2024

Fig. S8. SPTLC1 is required for metabolic reprograming in Th17 differentiation. (A) Heat map displaying the genes of gene set 'HALLMARK_MTORC1_SIGNALING' in WT and KO Th17 cells. **(B)** Heat map displaying the genes of gene set 'HALLMARK_MYC_TARGETS' in WT and KO Th17 cells. **(C)** Heat map displaying the genes of gene set 'SEMENZA_HIF1_TARGETS' in WT and KO Th17 cells



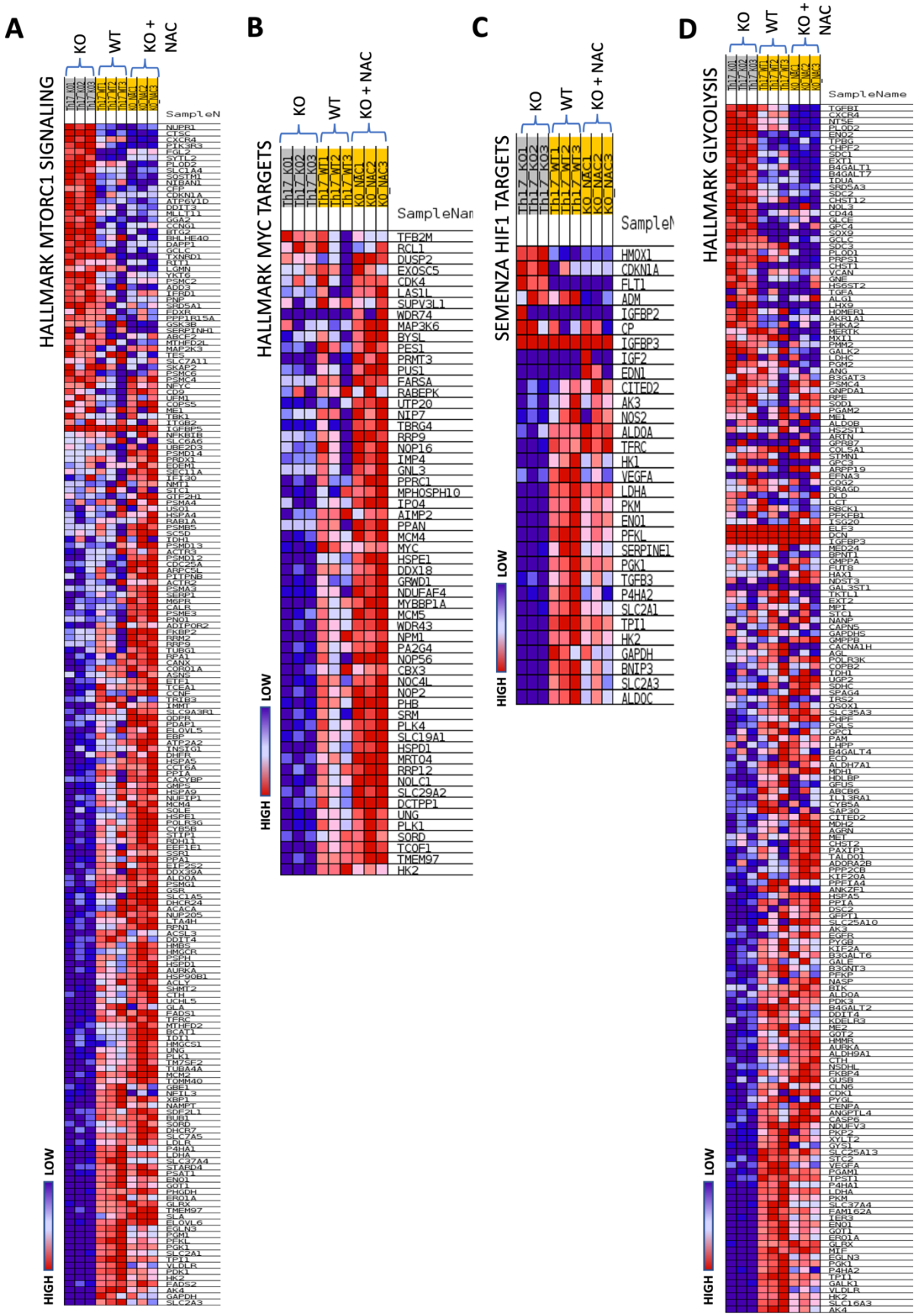
Supplementary Figure 9, Abimannan et al., 2024

Fig. S9. SPT enzyme product 3-KDS recues the defects in SPTLC1 deficient Th17 cells. (A) Heat map displaying the genes of gene set 'HALLMARK_MTORC1_SIGNALING' in WT, KO, and KO+KDS Th17 cells. **(B)** Heat map displaying the genes of gene set 'HALLMARK_MYC_TARGETS' in WT, KO, and KO+KDS Th17 cells. **(C)** Heat map displaying the genes of gene set 'SEMENZA_HIF1_TARGETS' in WT, KO, and KO+KDS Th17 cells. **(D)** Heat map displaying the genes of gene set 'HALLMARK_GLYCOLYSIS' in WT, KO, and KO+KDS Th17 cells.



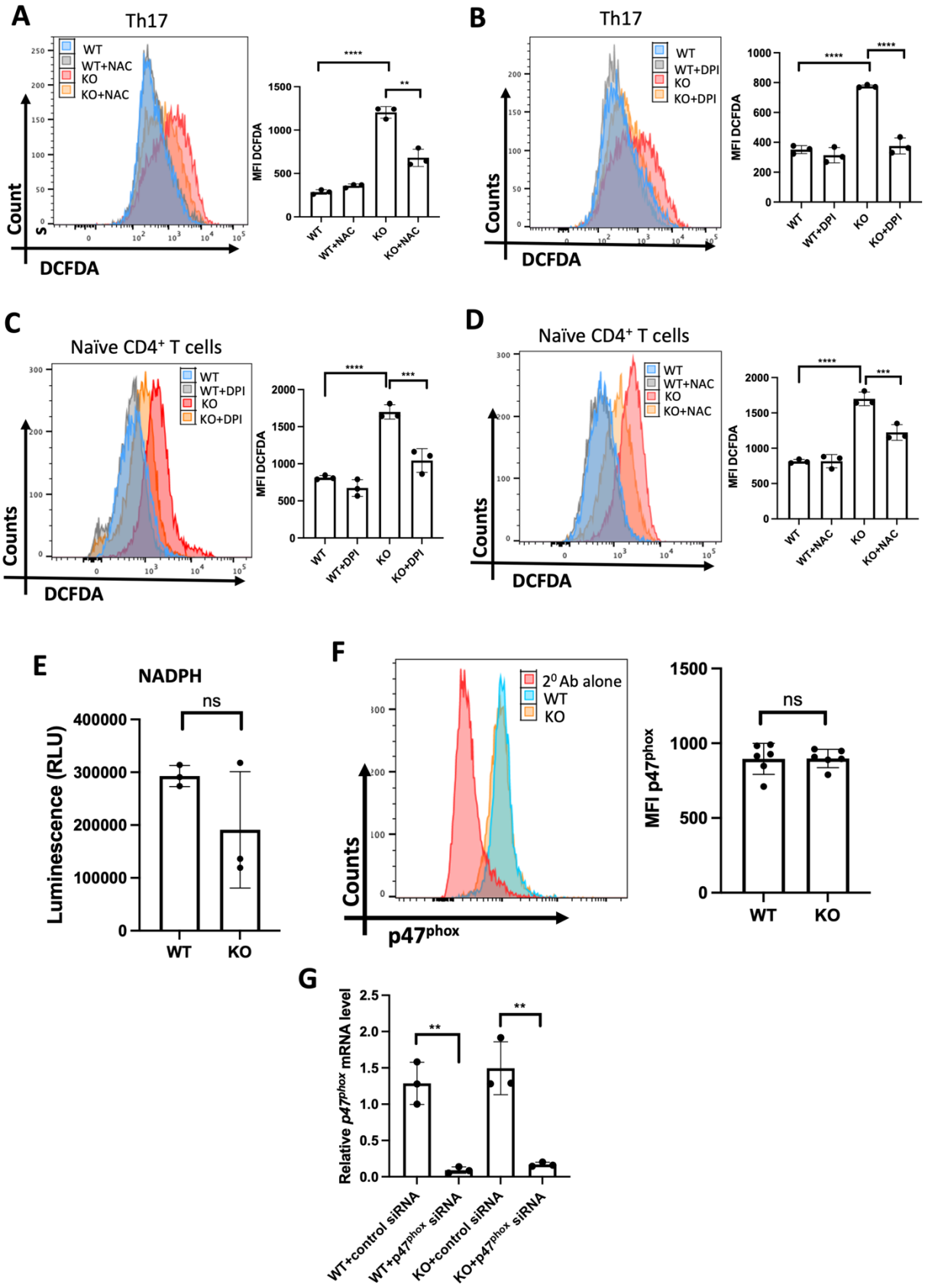
Supplementary Figure 10, Abimannan et al., 2024

Fig. S10. SPTLC1 deficiency impairs Th17 differentiation by increasing intracellular ROS. **(A)** Immunoblot of phospho-AMPK, total AMPK and β-actin in WT and KO naïve $CD4^+$ T cells differentiated for 12 hours under Th17 polarizing condition. Left panel shows representative western blot and the right panel is the quantitative data normalized to β-actin. n=3 biologically independent samples. **(B)** Gene Set Enrichment analysis (GSEA) result of "POSITIVE REGULATION OF REACTIVE OXYGEN SPECIES METABOLIC PROCESS (GO:2000379)" gene sets between WT and KO Th17 cells. **(C)** WT and KO naïve cells were differentiated under Th17 condition for 4 days with or without NAC and reactivated with PMA/Ionomycin for 6 hours and scored for intracellular cytokine by flow cytometry. Cumulative data from n=4-6 biologically independent samples. Each dot represents an individual mouse. Data presented as mean ± SEM: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ and ns, not significant.



Supplementary Figure 11, Abimannan et al., 2024

Fig. S11. SPTLC1 deficiency impairs Th17 differentiation by increasing intracellular ROS. **(A)** Heat map displaying the genes of gene set 'HALLMARK_MTORC1_SIGNALING' in WT, KO, and KO+NAC Th17 cells. **(B)** Heat map displaying the genes of gene set 'HALLMARK_MYC_TARGETS' in WT, KO, and KO+NAC Th17 cells. **(C)** Heat map displaying the genes of gene set 'SEMENZA_HIF1_TARGETS' in WT, KO, and KO+KDS Th17 cells. **(D)** Heat map displaying the genes of gene set 'HALLMARK_GLYCOLYSIS' in WT, KO, and KO+NAC Th17 cells.



Supplementary Figure 12, Abimannan et al., 2024

Fig. S12. SPTLC1 deficiency impairs Th17 differentiation by increasing intracellular ROS. **(A)** Naïve $CD4^+$ T cells from WT and KO mice were differentiated under Th17 condition for 3 days in presence or absence of NAC and cross-linked with anti-CD3 for 15 minutes with DCFDA dye and read in flow cytometry. Left panel is a representative flow figure and right panel shows the cumulative data of DCFDA MFI. n=3 biologically independent samples. **(B)** Naïve $CD4^+$ T from WT and KO mice were differentiated under Th17 condition for 3 days in presence or absence of DPI and cross-linked with anti-CD3 for 15 minutes with DCFDA dye and read in flow cytometry. Left panel is a representative flow figure and right panel shows the cumulative data of DCFDA MFI. n=3 biologically independent samples. **(C)** WT and KO naïve $CD4^+$ T cells were crosslinked with anti-CD3 for 15 minutes in the presence or absence of DPI with DCFDA dye and read in flow cytometry. Left panel is a representative flow figure and right panel shows the cumulative data of DCFDA MFI. n=3 biologically independent samples. **(D)** WT and KO naïve $CD4^+$ T cells crosslinked with anti-CD3 for 15 minutes in the presence or absence of NAC with DCFDA dye and read in flow cytometry. Left panel is a representative flow figure and right panel shows the cumulative data of DCFDA MFI. n=3 biologically independent samples. **(E)** Naïve $CD4^+$ T cells from WT and KO mice were differentiated under Th17 condition for 4 days and total cellular NADPH levels were determined by bioluminescent assay. Data presented as relative light unit (RLU). n=3 biologically independent samples. **(F)** WT and KO naïve $CD4^+$ T cells were crosslinked with anti-CD3 for 15 minutes and intracellular $p47^{phox}$ were detected by flow cytometry. n=3 biologically independent samples. **(G)** WT and KO Naïve $CD4^+$ T cells were nucleofected with control or p47phox siRNA. After 24 hours relative *p47phox* mRNA levels were determined by qPCR. n=3 biologically independent samples (Each dot in this panel represents cells pooled from 3 mice). Each dot in all other panel in this figure represents an individual mouse. All data presented as mean ± SEM: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$ and ns, not significant.