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SUPPLEMENTAL MATERIAL

Lim et al., http://www.jem.org/cgi/content/full/jem.20132378/DC1

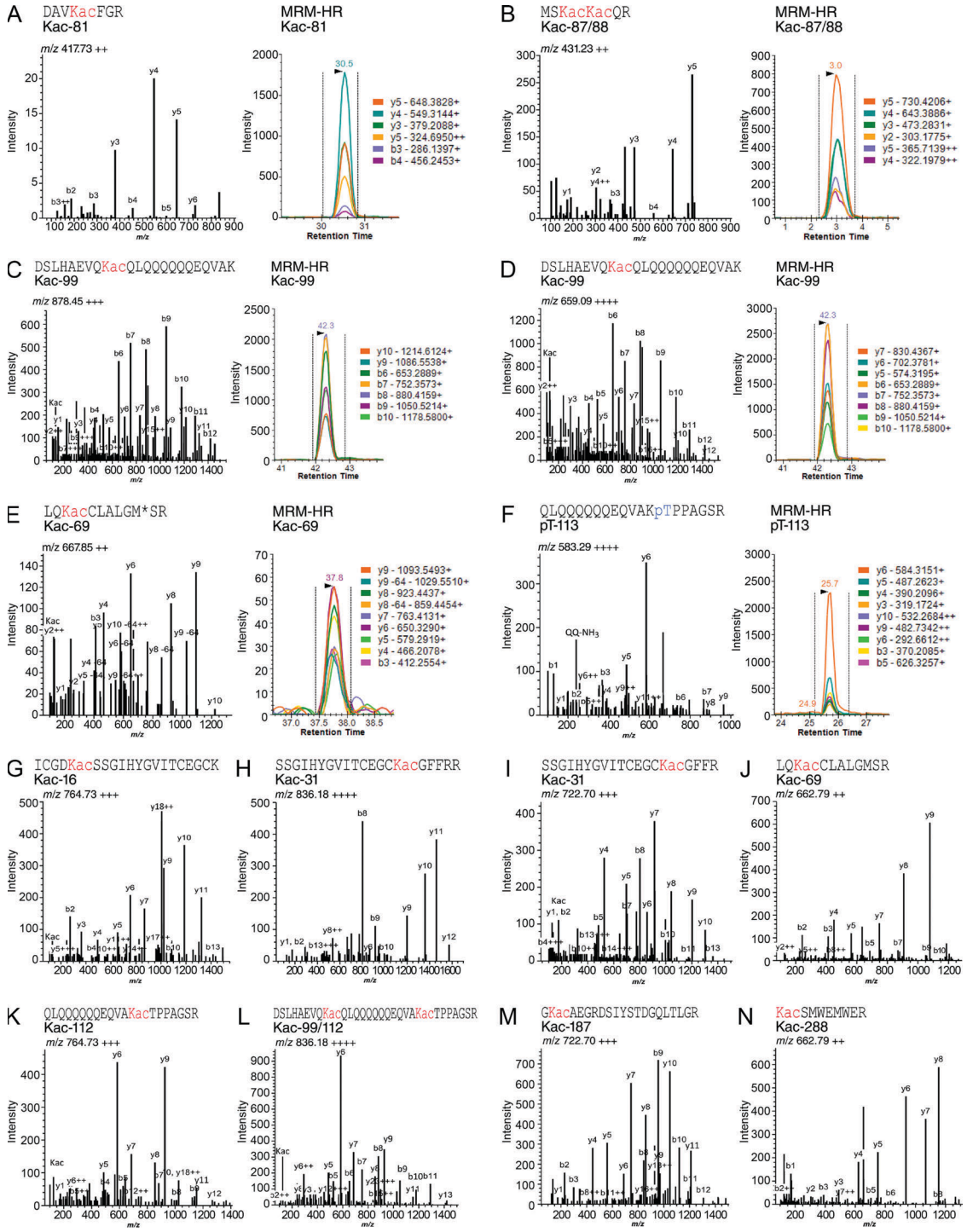

Figure S1. Tandem mass spectra (ESI-MS/MS) and identification of acetylated lysine residues in RORγt and corresponding MRM-HR assays. (A–F) MS/MS spectra and derived and optimized MRM-HR assays for acetylated peptides, specifically shown for (A) Kac-81 (DAVKacFGR, *m/z* at 417.73; z = 2), (B) Kac-87/88 (MSKacKacQR, *m/z* at 431.23; z = 2), (C) Kac-99 (DSLHAEVQKacQLQQQQQQEQVAK, *m/z* at 878.45; z = 3). (D) Kac-99 (DSLHAEVQKac-QLQQQQQQEQVAK, *m/z* at 659.09; z = 4), (E) Kac-69 (LQKacCLALGM^{ox}SR, *m/z* at 667.85; z = 2), and (F) pT-113, phosphopeptide QLQQQQQQEQVAKpTP-PAGSR, *m/z* at 583.29; z = 4). (G-N) Additional ESI-MS/MS spectra for acetylated peptide are displayed for (G) Kac-16 (ICGDKacSSGIHYGVITCEGCK, *m/z* 761.68; z = 3), (H) Kac-31 (SSGIHYGVITCEGCKacGFFRR, *m/z* 594.04; z = 4), (I) Kac-31 (SSGIHYGVITCEGCKacGFFR, *m/z* 739.68; z = 3), (J) Kac-69 (LQKac-CLALGMSR, *m/z* 659.85; z = 2), (K) Kac-112 (QLQQQQQQEQVAKacTPPAGSR, *m/z* 764.73; z = 3), (L) Kac-99&112 (DSLHAEVQKacQLQQQQQQEQ-VAKacTPPAGSR, *m/z* 836.18; z = 4), (M) Kac-187 (GKacAEGRDSIYSTDGQLTLGR, *m/z* 722.70; z = 3), and (N) Kac-288 (KacSMWEMWER, *m/z* 662.79; z = 2).

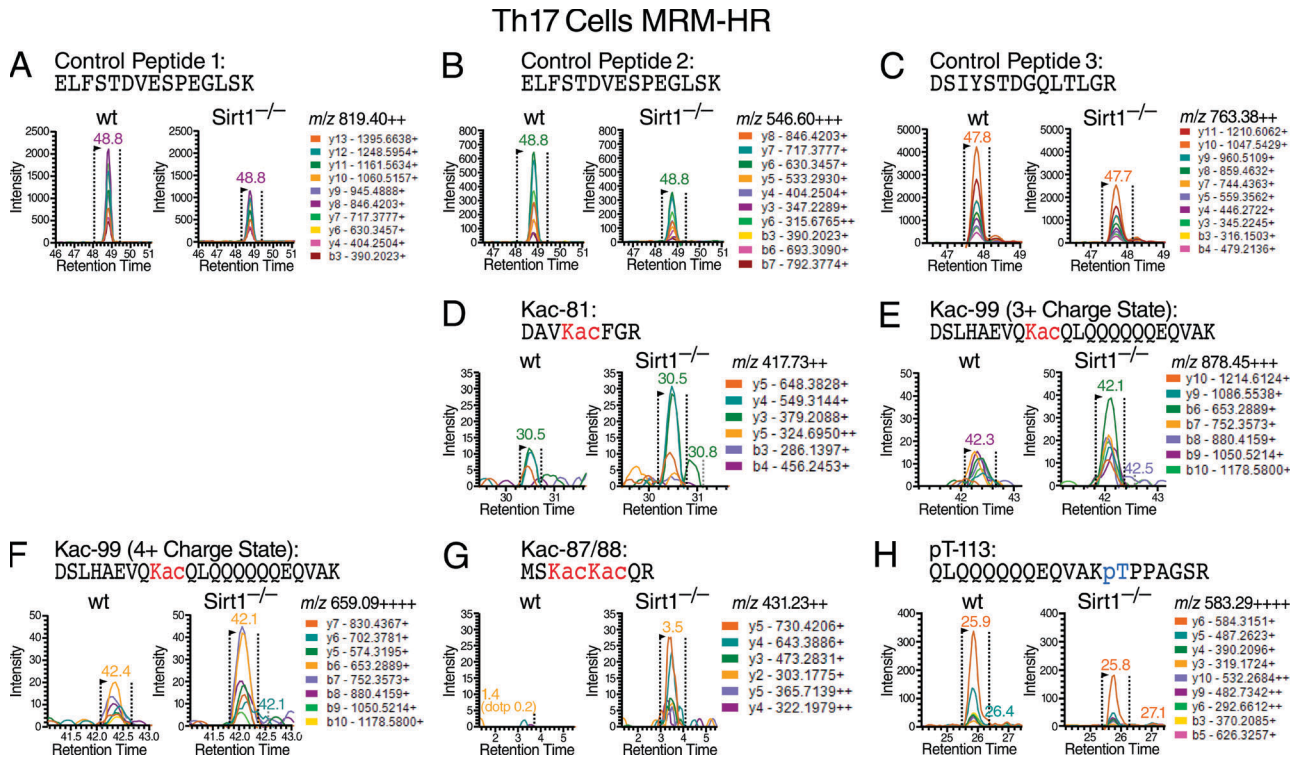

Figure S2. RORγt MRM-HR assays to monitor protein level and acetylation changes in Th17 cells from WT and Sirt1$^{-/-}$ mice. (A–C) Targeted MRM-HR assays were developed to monitor the protein level of RORγt in Th17 cells obtained from pools of 10 WT and 10 Sirt1$^{-/-}$ mice. Each sample was measured in technical duplicates. Three nonacetylated/unmodified peptide precursor ions were monitored for WT and Sirt1$^{-/-}$, respectively, (A) ELFSTDVESPEGLSK, *m/z* at 819.40^{2+}, (B) ELFSTDVESPEGLSK, *m/z* at 546.60^{+++}, and (C) DSIYSTDGQLTLGR, *m/z* at 763.38^{2+}. (D–I) To assess potential RORγt acetylation level changes at specific lysine residues in Th17 cells the acetylated peptide precursor ions were monitored for WT and Sirt1$^{-/-}$, respectively, (D) for Kac-81 (DAVKacFGR, *m/z* at 417.73; z = 2), (E) for Kac-99 (DSLHAEVQKacQLQQQQQQEQVAK, *m/z* at 878.45; z = 3), (F) for Kac-99 (DSLHAEVQKac-QLQQQQQQEQVAK, *m/z* at 659.09; z = 4), (G) for Kac-87/88 (MSKacKacQR, *m/z* at 431.23; z = 2), and (H) for pT-113 monitoring the corresponding phosphopeptide QLQQQQQQEQVAKpTPPAGSR, *m/z* at 583.29; z = 4).

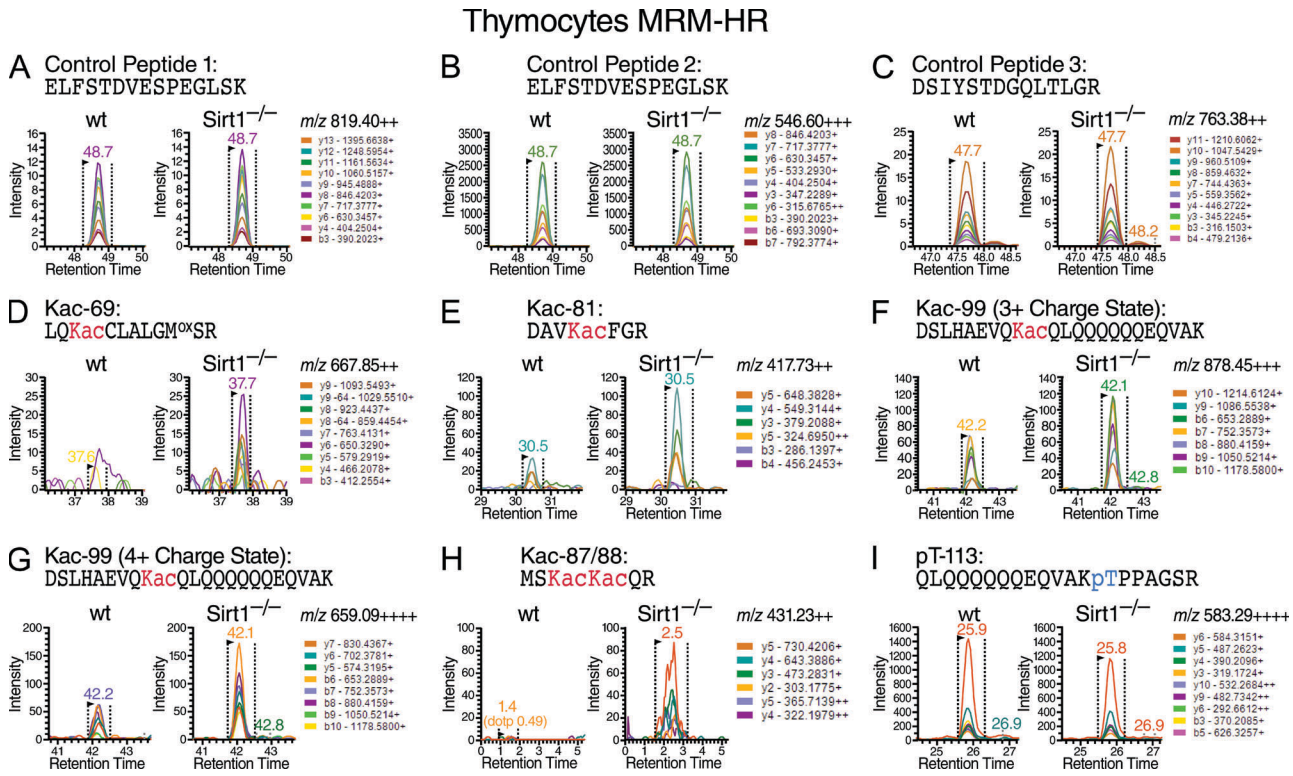

Figure S3. RORγt MRM-HR assays to monitor protein levels and acetylation changes in thymocytes from WT and Sirt1$^{-/-}$ mice. (A–C) Targeted MRM-HR assays were developed to monitor the protein level of RORγt in thymocytes obtained from pools of 10 WT and 10 Sirt1$^{-/-}$ mice. Each sample was measured in technical duplicates. Three nonacetylated/unmodified peptide precursor ions were monitored for WT and Sirt1$^{-/-}$, respectively, (A) ELFSTDVESPEGLSK, *m/z* at 819.40^{2+}, (B) ELFSTDVESPEGLSK, *m/z* at 546.60^{+++}, and (C) DSIYSTDGQLTLGR, *m/z* at 763.38^{2+}. (D–I) To assess potential RORγt acetylation level changes at specific lysine residues in thymocytes the acetylated peptide precursor ions were monitored for WT and Sirt1$^{-/-}$, respectively, (D) for Kac-69 (LQKacCLALGM^{ox}SR, *m/z* at 667.85; z = 2), (E) for Kac-81 (DAVKacFGR, *m/z* at 417.73; z = 2), (F) for Kac-99 (DSLHAEVQKac-QLQQQQQQEQVAK, *m/z* at 878.45; z = 3), (G) for Kac-99 (DSLHAEVQKacQLQQQQQQEQVAK, *m/z* at 659.09; z = 4), (H) for Kac-87/88 (MSKacKacQR, *m/z* at 431.23; z = 2), and (I) for pT-113 monitoring the corresponding phosphopeptide QLQQQQQQEQVAKpTPPAGSR, *m/z* at 583.29; z = 4).

Table S1, included as a separate Excel file, shows mass spectrometric identification details of RORγt. Table S2, included as a separate Excel file, shows overview and quantification/identification details of RORγt from WT and Sirt1$^{-/-}$ mice observed by MRM-HR.