YMTHE, Volume *31*

Supplemental Information

NAMPT is a metabolic checkpoint of IFNγ-producing CD4$^+$ T cells in lupus nephritis

Mengyuan Li, Yimei Lai, Binfeng Chen, Chaohuan Guo, Mianjing Zhou, Siyuan Zhao, Shuyi Wang, Jin Li, Niansheng Yang, and Hui Zhang

Supplementary Table1.**Demographics and clinical characteristics of SLE patients and healthy controls**.

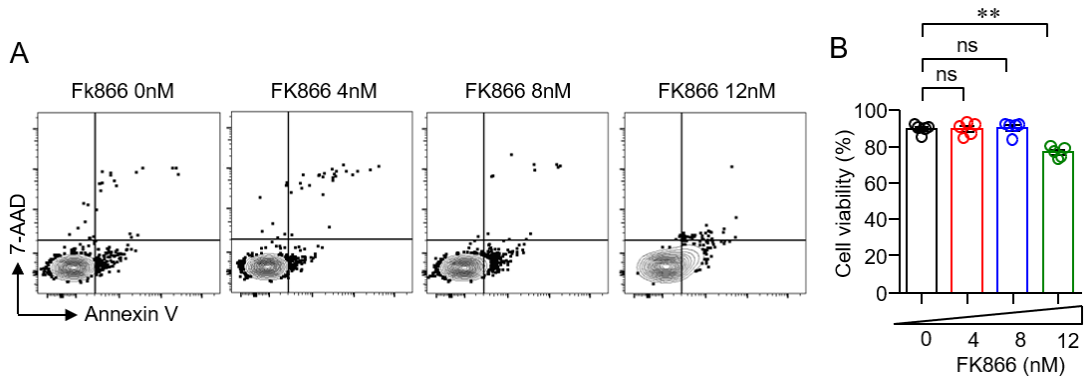
	SLE (N=110)	HC (N=95)
Age (year)	35.8 ± 13.2	31.3 ± 3.8
Gender (male/female)	8/102	16/79
SLEDAI	10.9 ± 6.2	/
ANA (IU/ml)	221.4 ± 109.0	/
Anti-dsDNA antibodies (IU/ml)	140.1 ± 130.7	/
ESR (mm/h)	47.2 ± 36.4	/
C3 (g/L)	0.6 ± 0.3	/
C4 (g/L)	0.1 ± 0.1	/
IgG (g/L)	14.2 ± 7.3	/
Serum Creatinine (μmol/L)	108.9 ± 123.7	/
White blood cells (10^9/L)	6.6 ± 3.5	/
Albumin (g/L)	31.7 ± 7.5	/
Platelet (10^{12}/L)	205.9 ± 86.6	/
Urinary proteins (g/24 h)	3.0 ± 4.2	/
Microscopic hematuria (no., RBC/HPF)*	53.5 ± 158.9	/
Medication (%)		/
Prednisone or methylprednisolone	100%	/
Mycophenolate Mofetil	34.9%	/
Cyclophosphamide	13.8%	/
Cyclosporin A	16.5%	/

Notes: All data are expressed as mean ± SD, except where specified. SLEDAI: Systemic Lupus Erythematosus Disease Activity Index, ANA: antinuclear antibodies, C3: Complement Component 3, C4: Complement Component 4. * red blood cells/high power field

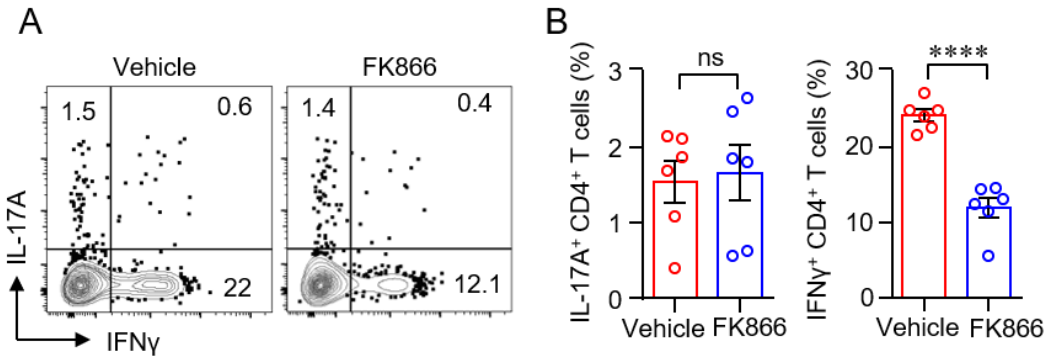
Supplementary Table 2. **Primers used in this study**.

Gene	Forward (5'-3')	Reverse (5'-3')
Ifng	TCGGTAACTGACTTGAATGTCCA	TCGCTTCCCTGTTTTAGCTGC
Srtuin 1	TAGCCTTGTCAGATAAGGAAGGA	ACAGCTTCACAGTCAACTTTGT
Sirtuin 2	TGCGGAACTTATTCTCCCAGA	GAGAGCGAAAGTCGGGGAT
Sirtuin 3	ACCCAGTGGCATTCCAGAC	GGCTTGGGGTTGTGAAAGAAG
Sirtuin 4	GCTTTGCGTTGACTTTCAGGT	CCAATGGAGGCTTTCGAGCA
Sirtuin 5	GCCATAGCCGAGTGTGAGAC	CAACTCCACAAGAGGTACATCG
Sirtuin 6	CCCACGGAGTCTGGACCAT	CTCTGCCAGTTTGTCCCTG
Sirtuin 7	GACCTGGTAACGGAGCTGC	CGACCAAGTATTTGGCGTTCC
β-actin	GATCATTGCTCCTCCTGAGC	CGTCATACTCCTGCTTGCTG

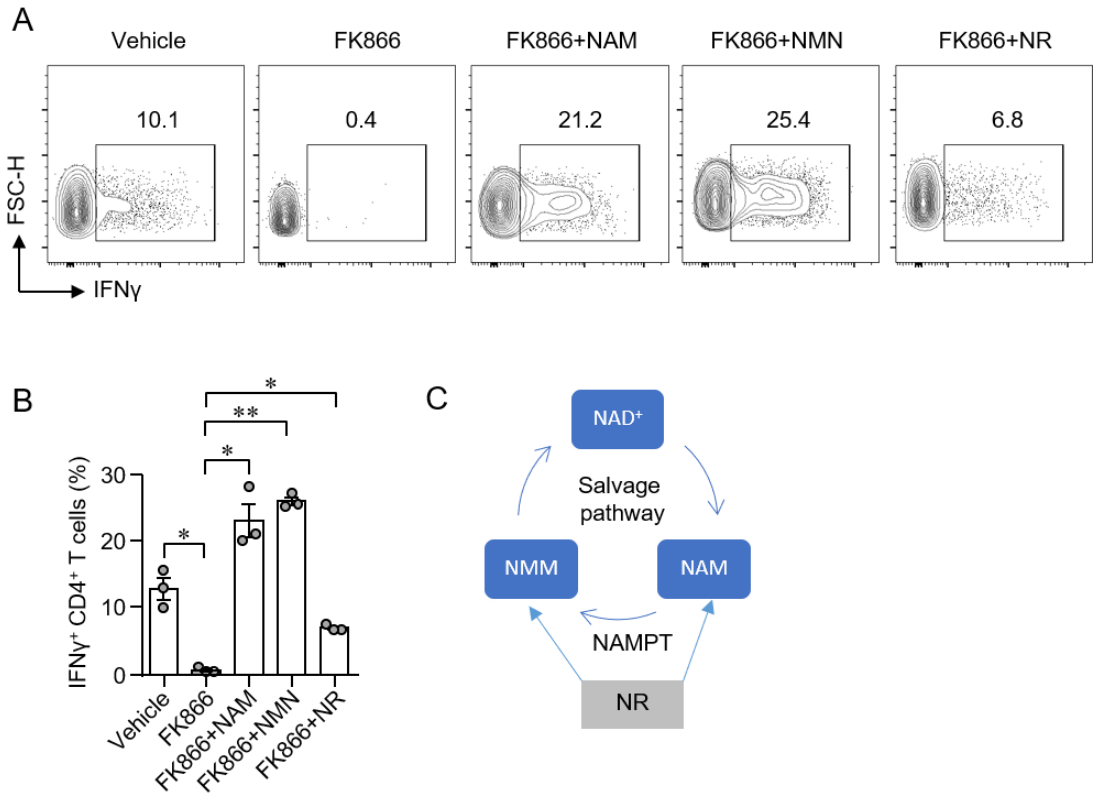
Supplementary Figures and legends



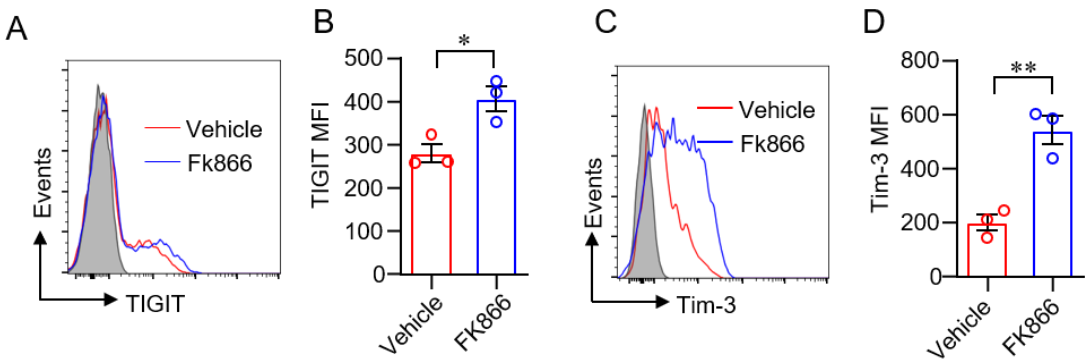
Supplementary Figure 1**. FK866 showed no effects on cell death of CD4⁺ T cells.** (A, B) Human CD4⁺ T cells were stimulated with anti-CD3/CD28 beads in the presence of different concentrations of FK866 as indicated for 3 d. Cell viability was evaluated for the expression of 7-AAD and Annexin V by flow cytometry. Experiment was repeated 5 times. Data are mean ± SEM. ***p*<0.01 by one-way ANOVA followed by adjustments for multiple comparison. ns: not significant.



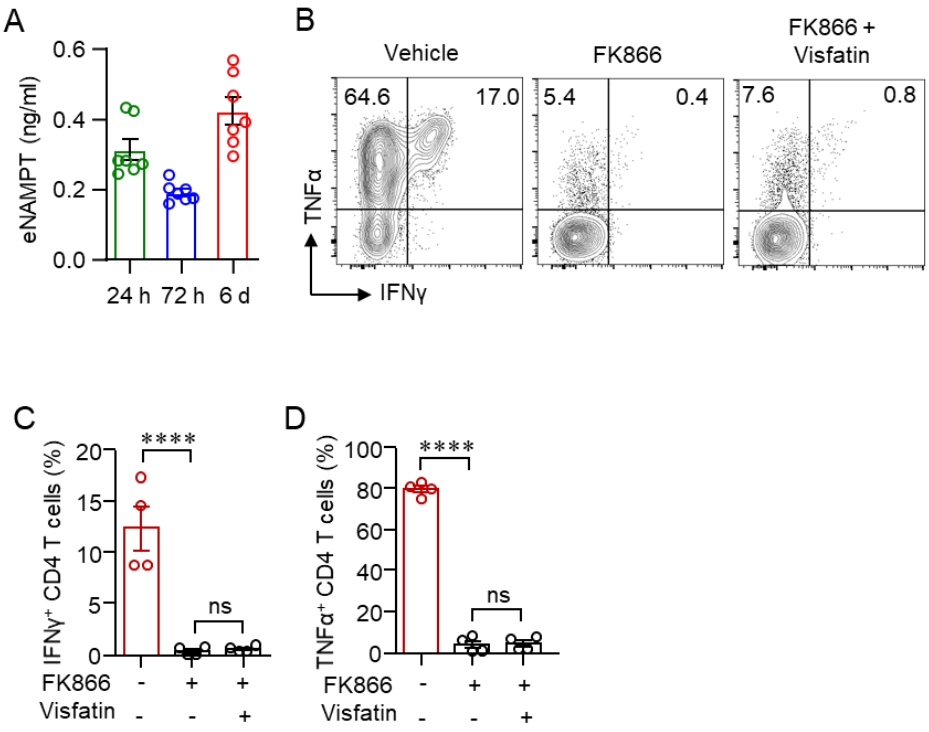
Supplementary Figure 2. **FK866 inhibits IFNγ production in T cells.** (A, B) PBMCs were stimulated with OKT3 in the presence or absence of FK866 (8 nM) for 6 d. IFNγ and IL-17A expression in $CD4^+$ T cells were detected by flow cytometry. Data from 6 independent samples. Data are mean ± SEM. ****p<0.0001 by Student's t test. ns: not significant.



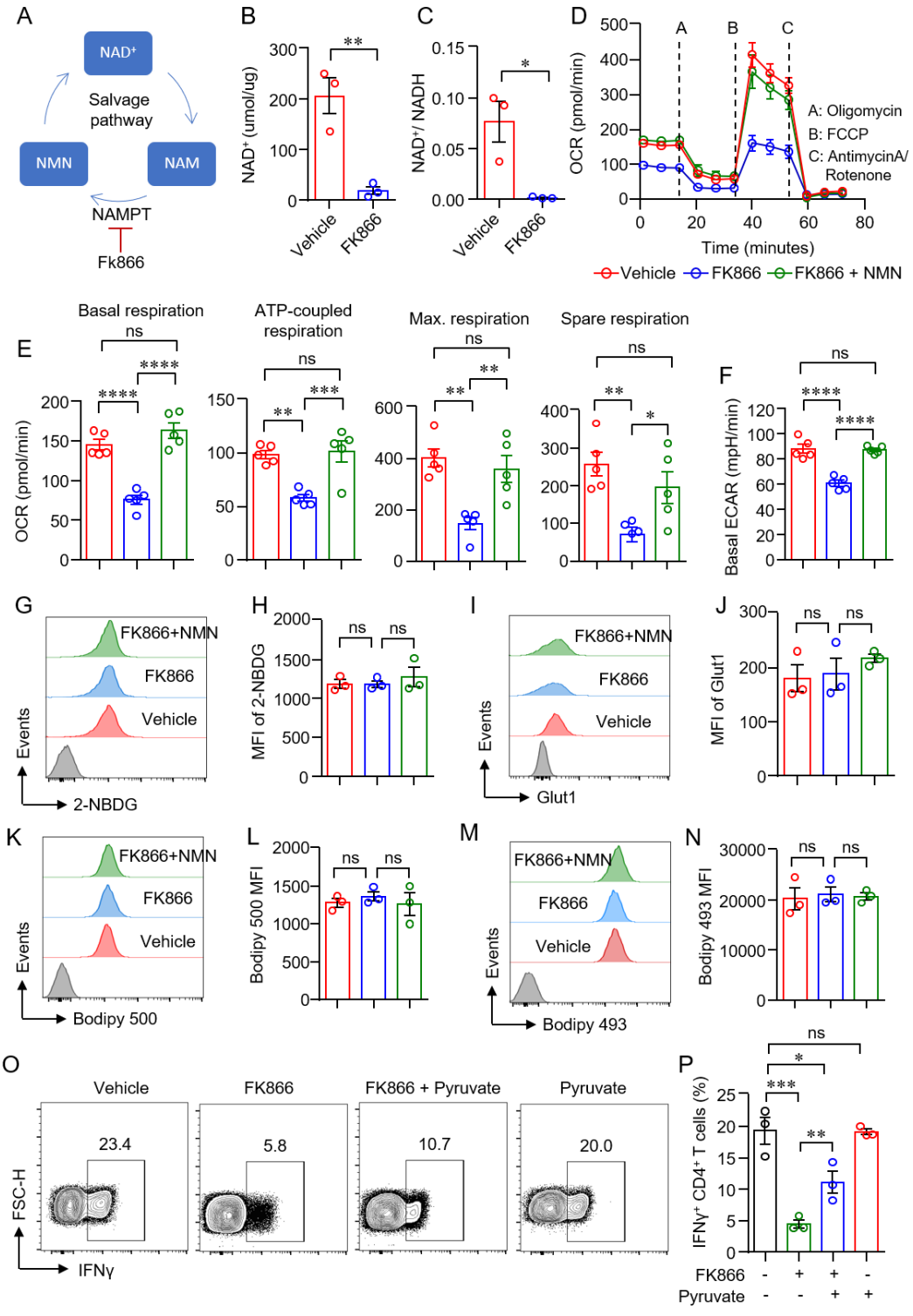
Supplementary Figure 3. **NAMPT-mediated NAD⁺ synthesis is required for IFNγ production in T cells.** (A, B) CD4⁺ T cells were stimulated with anti-CD3/CD28 beads in the presence or absence of FK866 (8 nM). NAM (1 μM), NMN (1 mM) or NR (1 μM) was included in some of the experiments. IFNγ expression was determined by flow cytometry. Data from 3 independent samples. (C) Salvage pathway of NAD⁺ metabolism. Data are mean ± SEM. *p<0.05, **p<0.01 by one-way ANOVA followed by adjustments for multiple comparisons.



Supplementary Figure 4. **FK866 induced TIGIT and Tim-3 expression.** $CD4^+$ T cells were stimulated with anti-CD3/CD28 beads in the presence or absence of FK866 (8 nM). (A, B) TIGIT expression in $CD4^+$ T cells were detected by flow cytometry. Data from 3 independent samples. (C, D) Tim-3 expression in $CD4^+$ T cells were detected by flow cytometry. Data from 3 independent samples. Data are mean ± SEM. *p<0.05, **p<0.01 by Student's t test.

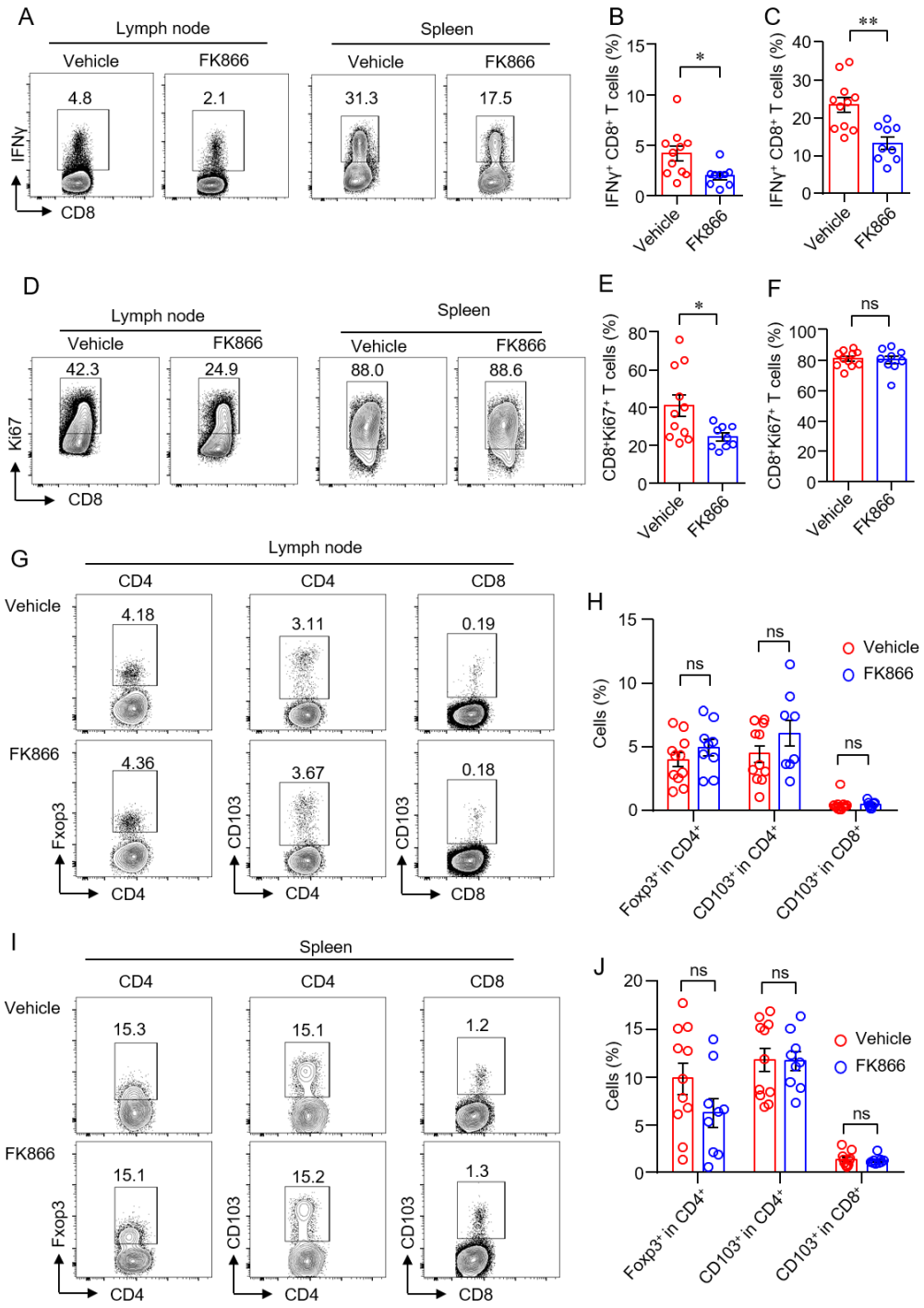


Supplementary Figure 5. **Extracellular NAMPT could not reverse FK866's inhibition on CD4⁺ T cells.** (A) CD4⁺ T cells were stimulated with anti-CD3/CD28 beads. The concentration of extracellular NAMPT (eNAMPT) in the supernatant of CD4⁺ T cells at different time points were measured by ELISA. (B-D) CD4⁺ T cells were stimulated with anti-CD3/CD28 beads for 3 d. FK866 and Visfatin (200 ng/ml) were included during cell cultured as indicated. IFNγ and TNFα expression was measured by flow cytometry (n=4). **** p<0.0001 by one-way ANOVA followed by adjustments for multiple comparisons. ns: not significant.



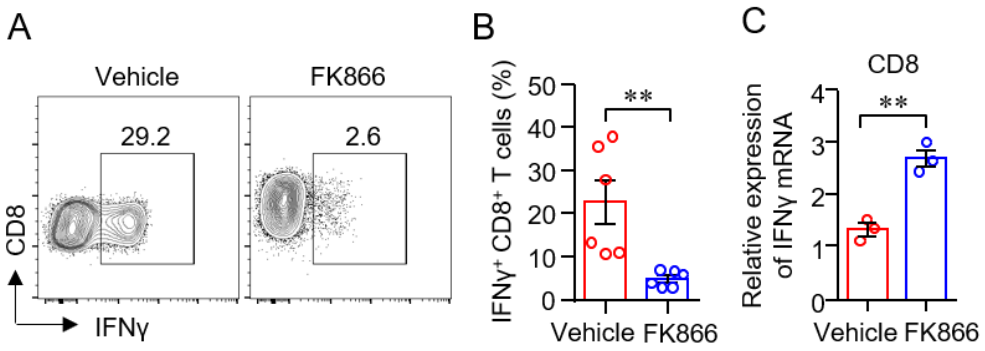
Supplementary Figure 6. **FK866 suppressed cellular energy metabolism in CD4⁺ T cells independent of glucose and lipid uptake**. (A) Graph illustrated NAMPT in NAD⁺ salvage pathway. (B, C) CD4⁺ T cells were stimulated with

anti-CD3/CD28 beads in the presence or absence of FK866 for 3 d. NAD^+/NADH in $CD4^+$ T cells was measured using a WST-8 based colorimetric assay (n=3). (D-F) $CD4^+$ T cells were stimulated with anti-CD3/CD28 beads in the presence of FK866. NMN (1 mM) was added to reverse FK866. Metabolic activities were measured by a Seahorse XF96 analyzer. Oxygen consumption rate (OCR) of basal respiration, respiration coupled to ATP production, maximal (Max.) respiration, spared respiratory capacity and basal extracellular acidification rates (ECAR) were summarized from 5 independent samples. (G-J) Human $CD4^+$ T cells were stimulated with anti-CD3/CD28 beads in the presence of FK866 (8 nM) for 3 d. Uptake of 2-NBDG (G, H) and Glut1 expression (I, J) were measured by flow cytometry. Data from 3 independent samples. (K-N) Cells were incubated with Bodipy 493 (lipid uptake) and Bodipy 500 (lipid content) for lipid measurement by flow cytometry (n=3). (O, P) Human $CD4^+$ T cells were stimulated with anti-CD3/CD28 beads in the presence of FK866 (8 nM) for 3 d. Pyruvate (2 μM) was added in some of the experiments. IFNγ expression was measured by flow cytometry. Data are mean ± SEM. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by one-way ANOVA followed by adjustments for multiple comparisons in panels E, F, H, J, L, N and P and Student's test in panels B and C. ns: not significant.

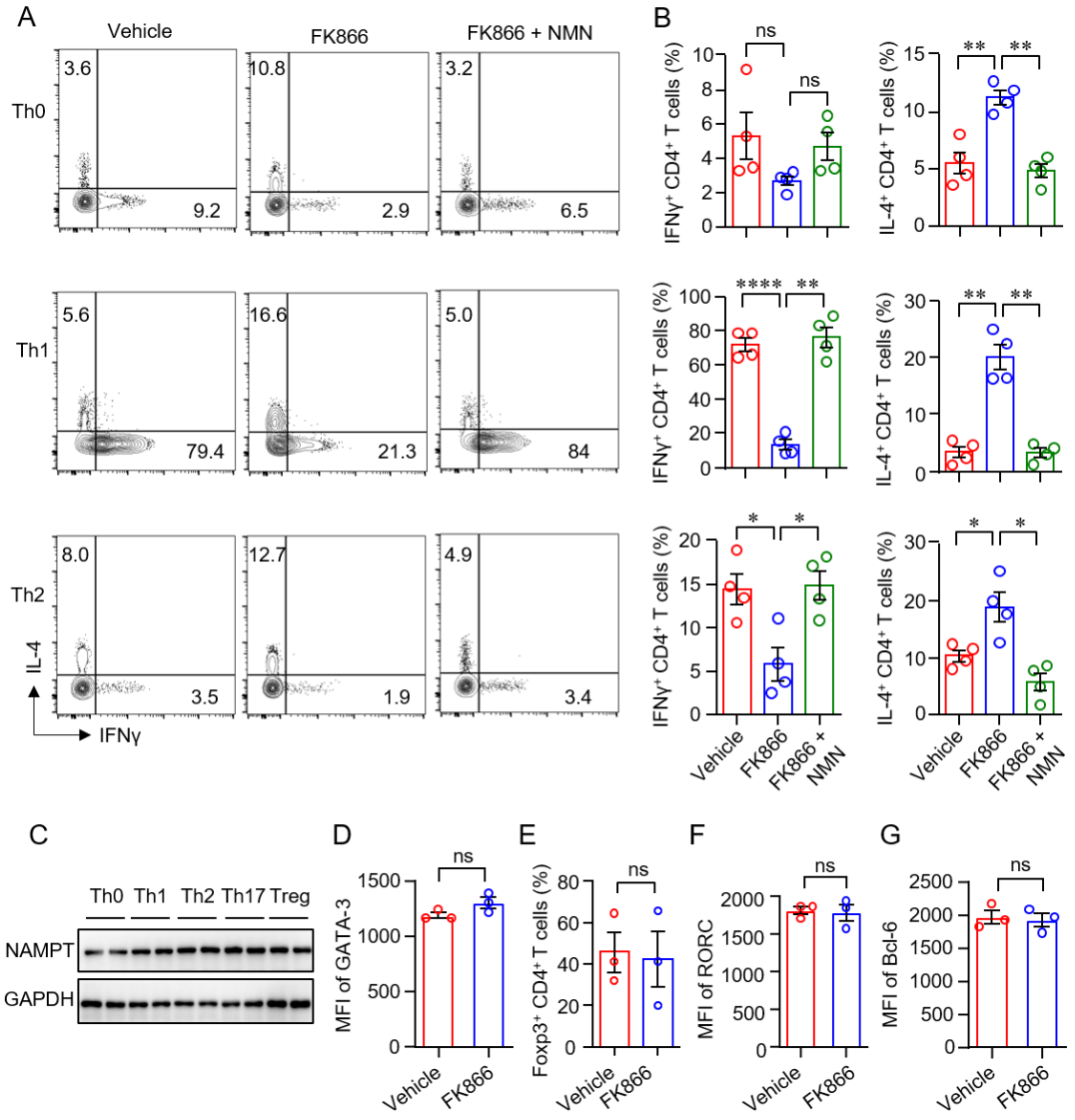


Supplementary Figure 7. **Impacts of FK866 on T cells in the lymphoid organs of MRL/*lpr* mice**. MRL/*lpr* mice were treated with FK866 as in Fig. 6A. Single cell suspension were prepared from spleens or kidney draining lymph nodes (DLN). (A-F) IFNγ and Ki-67 expression in $CD8^+$ T cells from spleen or kidney DLN was measured by flow cytometry. (G-J) Foxp3 and CD103

expression in $CD4^+$ and $CD8^+$ T cells from kidney DLN or spleen were measured by flow cytometry. Data are mean ± SEM. *p<0.05, **p<0.01 by Student's t test. ns: not significant.

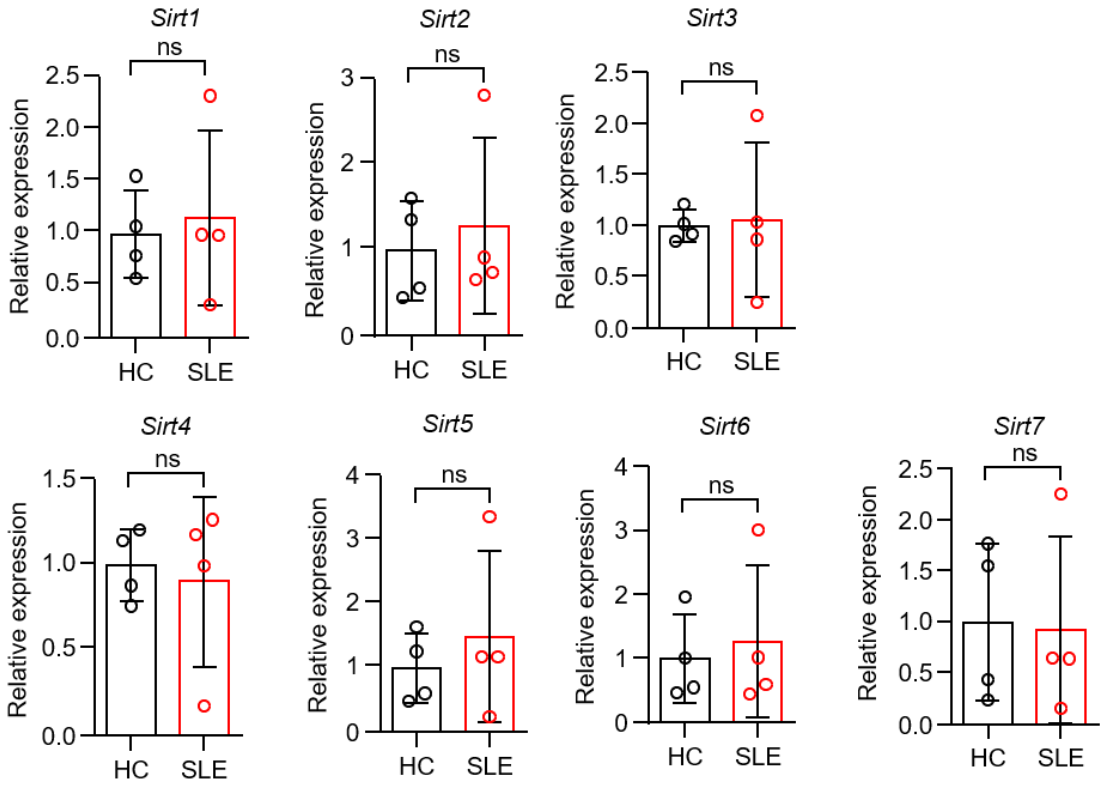


Supplementary Figure 8. **FK866 inhibited IFNγ production but induced *Ifng* mRNA**. $CD8^+$ T cells were stimulated with anti-CD3/CD28 beads for 6 d. FK866 was added to the culture 4 h before stimulation with ionomycin, PMA and BFA. IFNγ expression was measured by flow cytometry (A, B) and qPCR (C). Data are mean ± SEM. $^{**}p<0.01$ by Student's t test.



Supplementary Figure 9. **NAMPT in CD4⁺ T cell differentiation**. Naïve human CD4⁺ T cells were stimulated with anti-CD3/CD28 beads under different Th cell differentiation conditions. (A, B) Naïve CD4⁺ T cells were cultured under Th1 and Th2 differentiation conditions for 6 d. IFNγ and IL-4 expression in CD4⁺ T cells were measured by flow cytometry. Data from 4 independent samples. (C-G) Naïve CD4⁺ T cells were cultured under Th0, Th1, Th2, Th17, Treg cell differentiation conditions for 6 d. (C) NAMPT expression was measured by western blot. Representative bands of 4 samples. (D-G) GATA-3, Foxp3, RORC

and Bcl-6 expression in $CD4^+$ T cells were measured by flow cytometry. Data from 3 independent samples. Data are mean ± SEM. *p<0.05, **p<0.01, ****p<0.0001 by one-way ANOVA followed by adjustments for multiple comparisons in panel B and Student's t test in panels D-G. ns: not significant.



Supplementary Figure 10. **Sirtuins in CD4⁺ T cells were not different between healthy controls and patients with SLE**. CD4⁺ T cells were isolated from patients with SLE or HC. Transcripts of Sirt1, Sirt2, Sirt3, Sirt4, Sirt5, Sirt6, and Sirt7 in CD4⁺ T cells were measured by qPCR. n=4. ns: not significant by Student's t test.