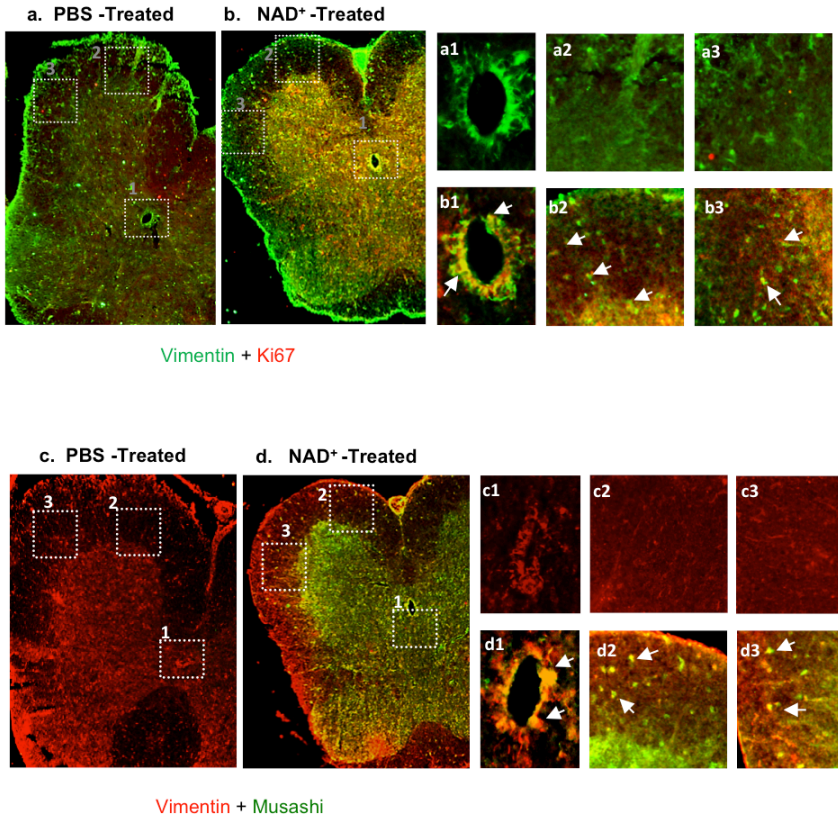
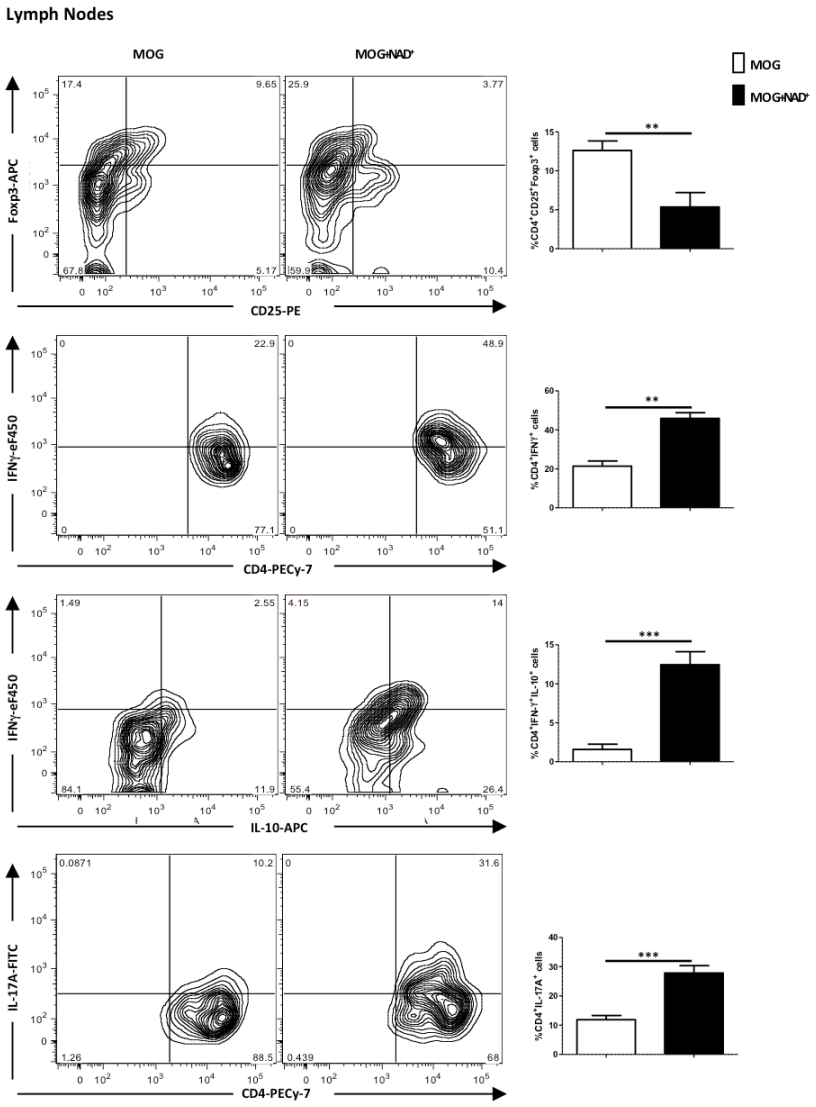
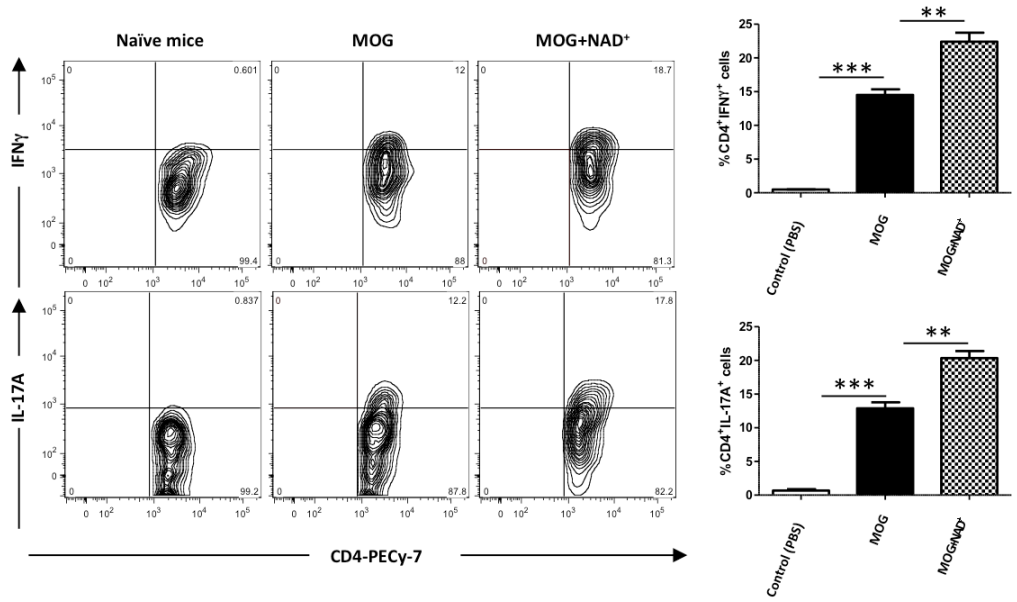


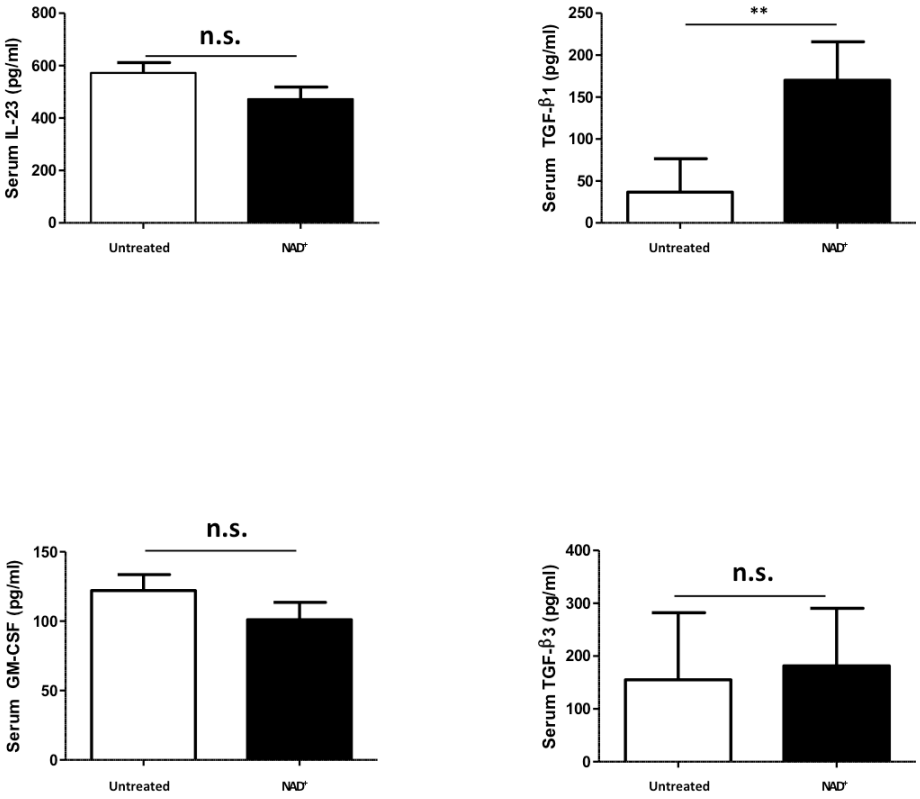
Supplementary Figure 1: NAD^+ enhances ependymal cell proliferation and Musashi expression. NAD^+ treatment significantly increased Ki67 **(b)** and Musashi **(d)** labeling in ependymal cell population (1), subependymally and in outer circumference of the spinal cord (2 & 3) when compared to controls **(a, c)**. Boxed regions in **a-d** have been magnified in a 1-d3 and white arrows depict increased stem cell proliferation. Scale bars: a, 100 µm (applies to b-d); a1, 20 µm (applies to a2-d3).



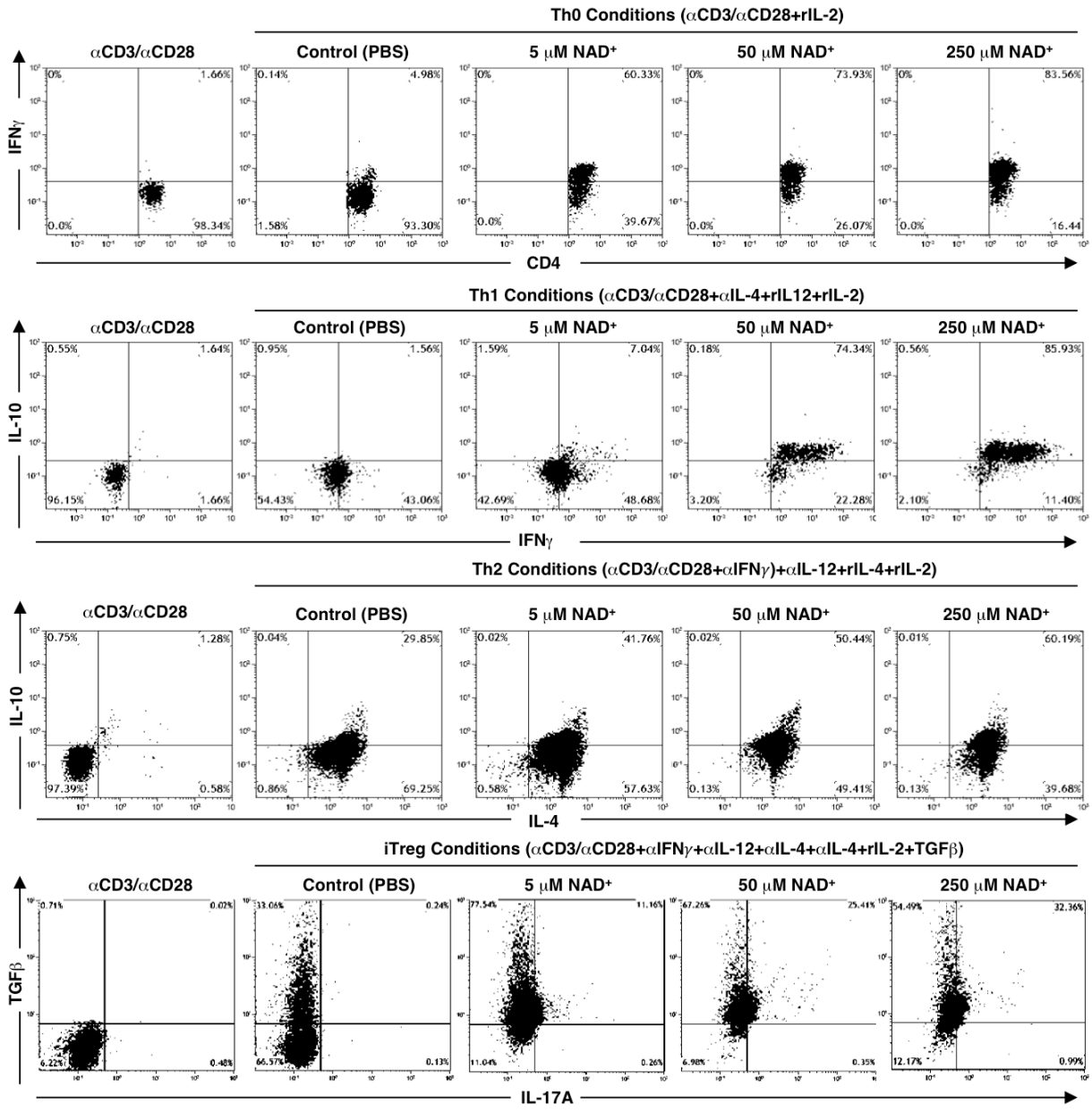
Supplementary Figure 2: NAD$^+$ alters immune response in the lymph nodes. Wild type C57BL/6 mice were subjected to EAE by MOG immunization and treated daily with intraperitoneal injection of 60mg of NAD$^+$ or a placebo solution (PBS). After 18 days mice were euthanized and CD4$^+$ T cells were isolated from lymph nodes and stimulated with PMA/ionomycin. Frequencies of CD4$^+$IFNγ$^+$, CD4$^+$IFNγ$^+$IL-10$^+$ and CD4$^+$IL-17A$^+$cells were analyzed by flow cytometry. (n=5) $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, n.s., not significant, as determined by Student's t-test, comparing the indicated groups.



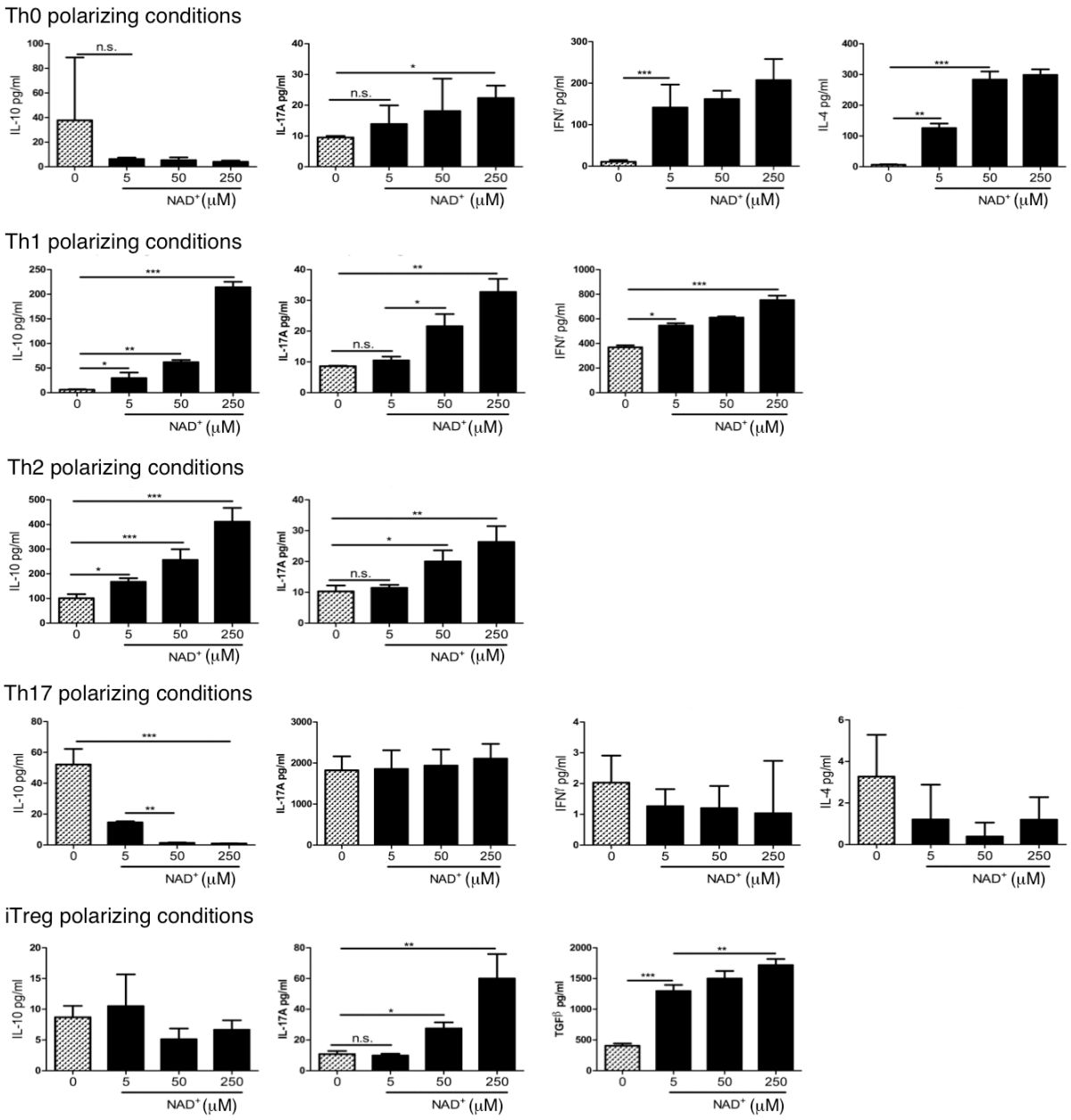
Supplementary Figure 3: NAD^+ increases systemically $CD4^+IFN\gamma^+$ and $CD4^+IL\text{-}17A^+$ cells. Wild type C57BL/6 mice were subjected to EAE by MOG immunization and treated daily with intraperitoneal injection of 60mg of NAD^+ or a placebo solution (PBS). After 18 days mice were euthanized and $CD4^+$ T cells were isolated from spleens and stimulated with PMA/ionomycin and frequencies of $CD4^+IFN\gamma^+$ and $CD4^+IL\text{-}17A^+$ cells were analyzed by flow cytometry. (n=5) $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, n.s., not significant, as determined by Student's t-test, comparing the indicated groups.



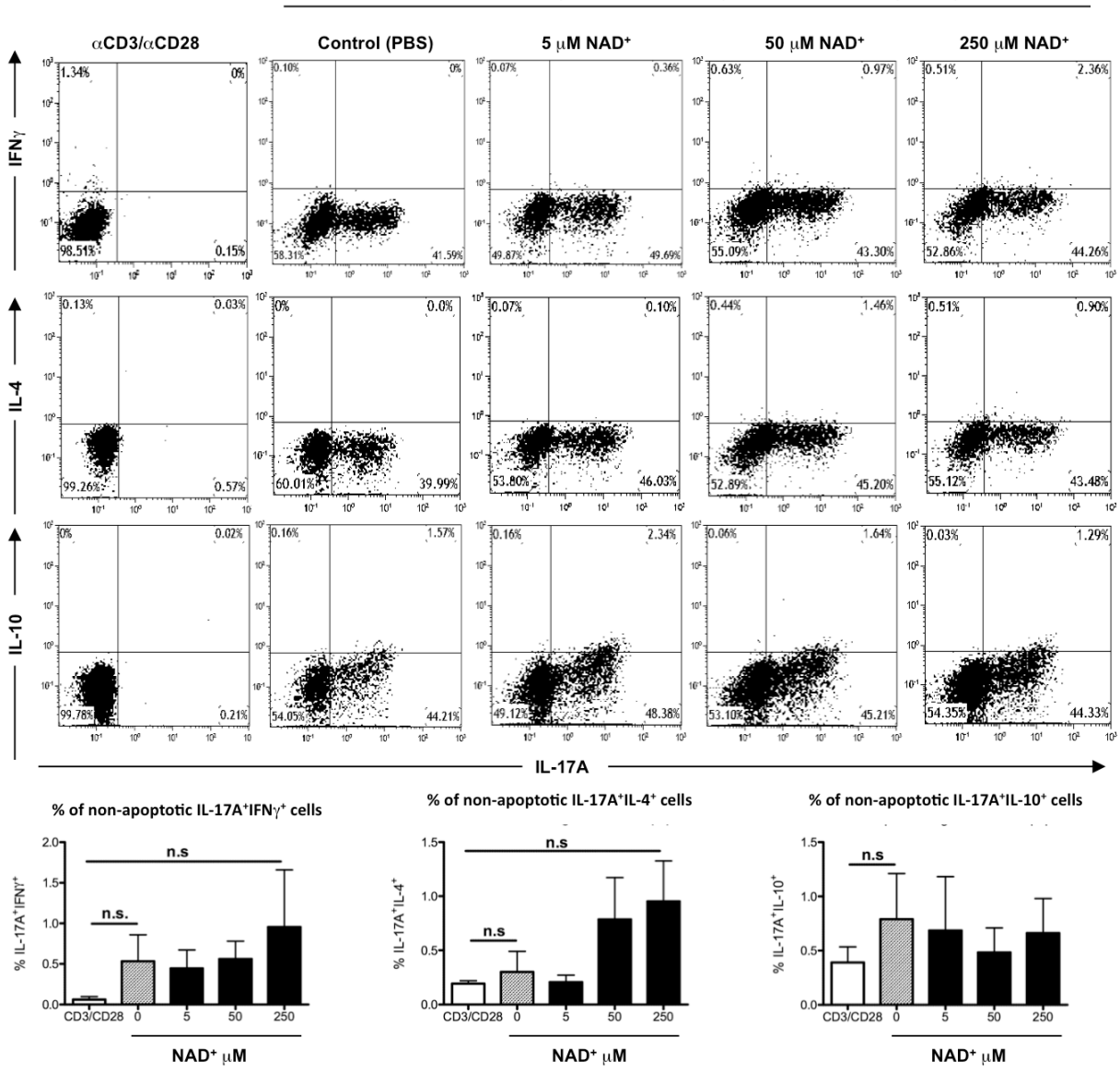
Supplementary Figure 4: NAD^+ induces a modest systemic increase of $TGF\beta_1$ but not other cytokines. Wild type C57BL/6 mice were subjected to EAE by MOG immunization and treated daily with intraperitoneal injection of 60mg of NAD^+ or a placebo solution (PBS). After 18 days mice were euthanized and serum levels of IL-23, $TGF\beta_1$, GM-CSF and $TGF\beta_3$ cytokines were assessed by ELISA. (n=5) **, $p < 0.01$; n.s., not significant, as determined by Student's t-test, comparing the indicated groups.



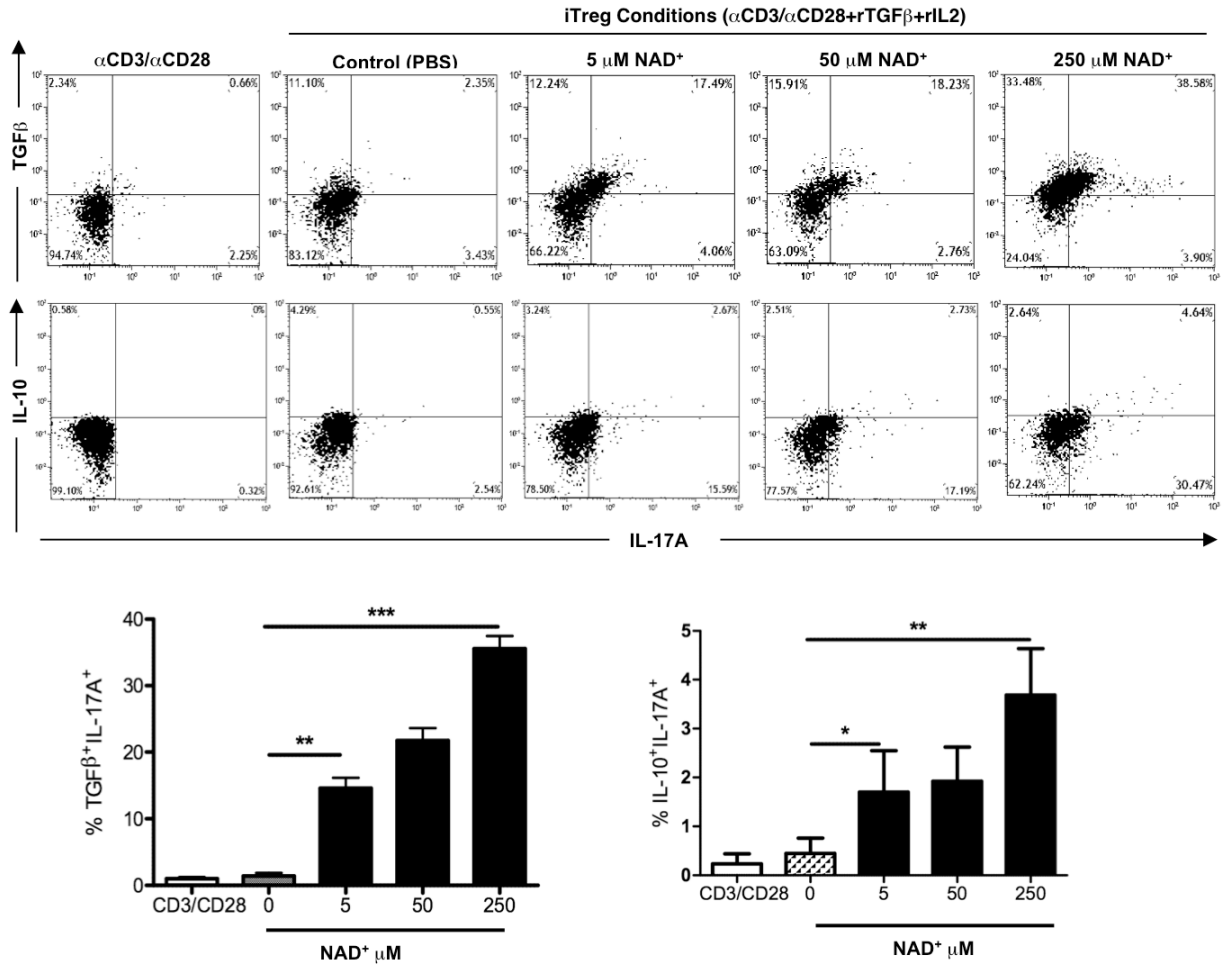
Supplementary Figure 5: NAD$^+$ alters CD4$^+$ T cell differentiation of sorted naive CD4$^+$CD25$^-$CD44^{low}CD62L^{high} cells from wild type mice in different polarizing conditions. Sorted naïve CD4$^+$CD25$^-$CD44^{low}CD62L^{high} T cells were isolated from spleens of wild type mice (C57BL/6 background) and activated with α-CD3/α-CD28 antibodies only or in Th0 (recombinant IL-2 cytokine at a concentration of 50ng/ml), Th1, Th2 or iTreg-polarizing conditions. Cells that were cultured in Th0, Th1, Th2, and iTreg polarizing conditions were stimulated with increasing concentrations of NAD$^+$ (0, 5, 50 and 250μM). After 96 hours, cell frequency of CD4$^+$IL-IFNγ^+ in Th0, CD4$^+$IL-IFNγ^+IL-10$^+$ in Th1, CD4$^+$ IL-4$^+$IL-10$^+$ in Th2 and CD4$^+$IL-17A$^+$TGFβ$^+$ in iTreg conditions was measured by flow cytometry by gating on non-apoptotic CD4$^+$ cells. To set the gates, flow cytometry dot plots were based on comparison with isotype controls, fluorescence minus one (FMO), permeabilized and unpermeabilized unstained cells (n=15; the data derived from three different experiments). n.s.; not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, as determined by Student's t-test, comparing the indicated groups.



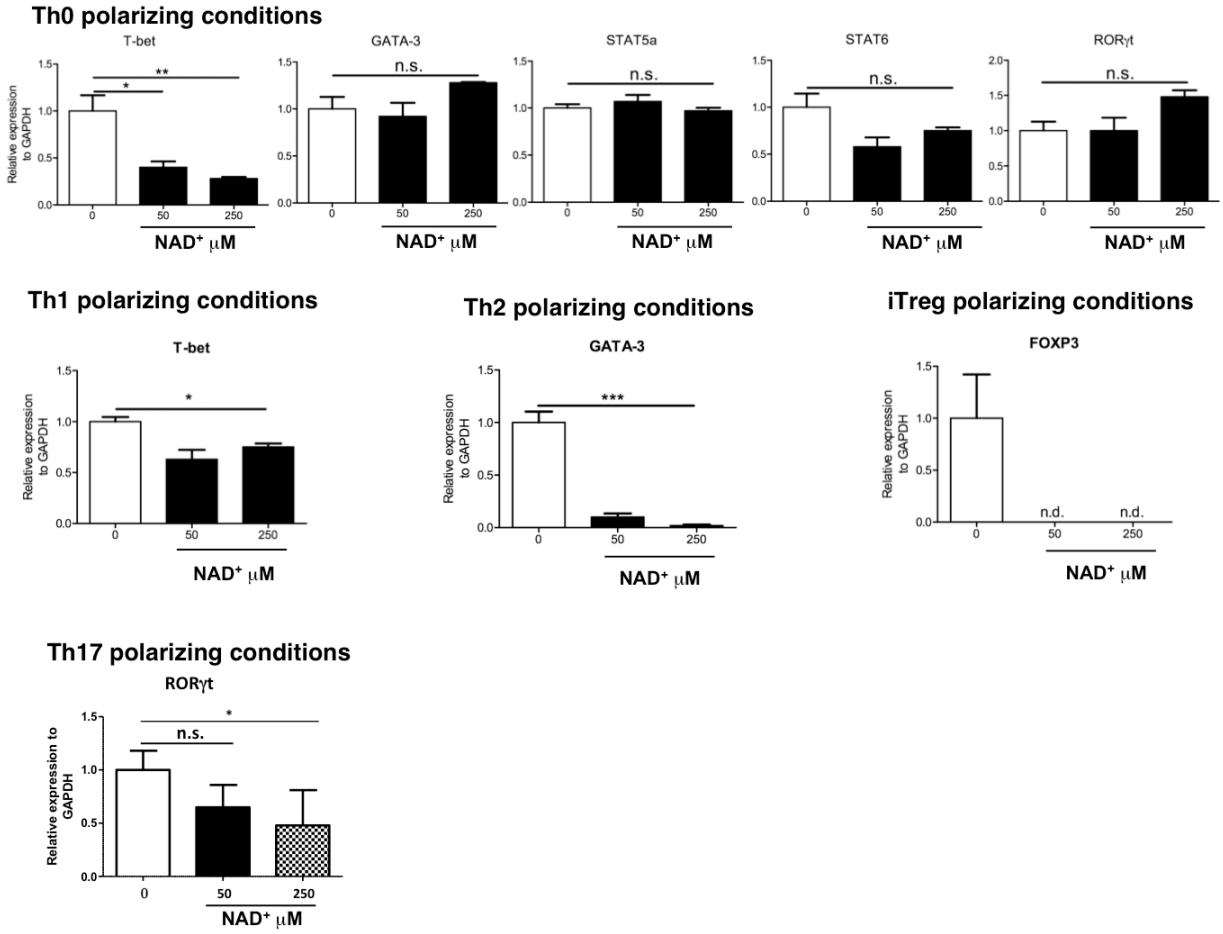
Supplementary Figure 6: NAD^+ regulates cytokine secretion in different polarizing conditions but not in Th17 conditions. Sorted naïve $CD4^+$ T cells were isolated from spleens of 5C.C7 *Rag2*$^{-/-}$ mice (C57BL/6 background) and activated with α-CD3/α-CD28 antibodies only or in Th0 (recombinant IL-2 cytokine at a concentration of 50ng/ml), Th1, Th2, Th17 or iTreg-polarizing conditions. Cells that were cultured in Th0, Th1, Th2, Th17 and iTreg polarizing conditions were stimulated with increasing concentrations of NAD^+ (0, 5, 50 and 250μM). After 96 hours, cytokine secretion was assessed by ELISA (n=15; the data derived from three different experiments). n.s.; not significant; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, as determined by Student's t-test, comparing the indicated groups.



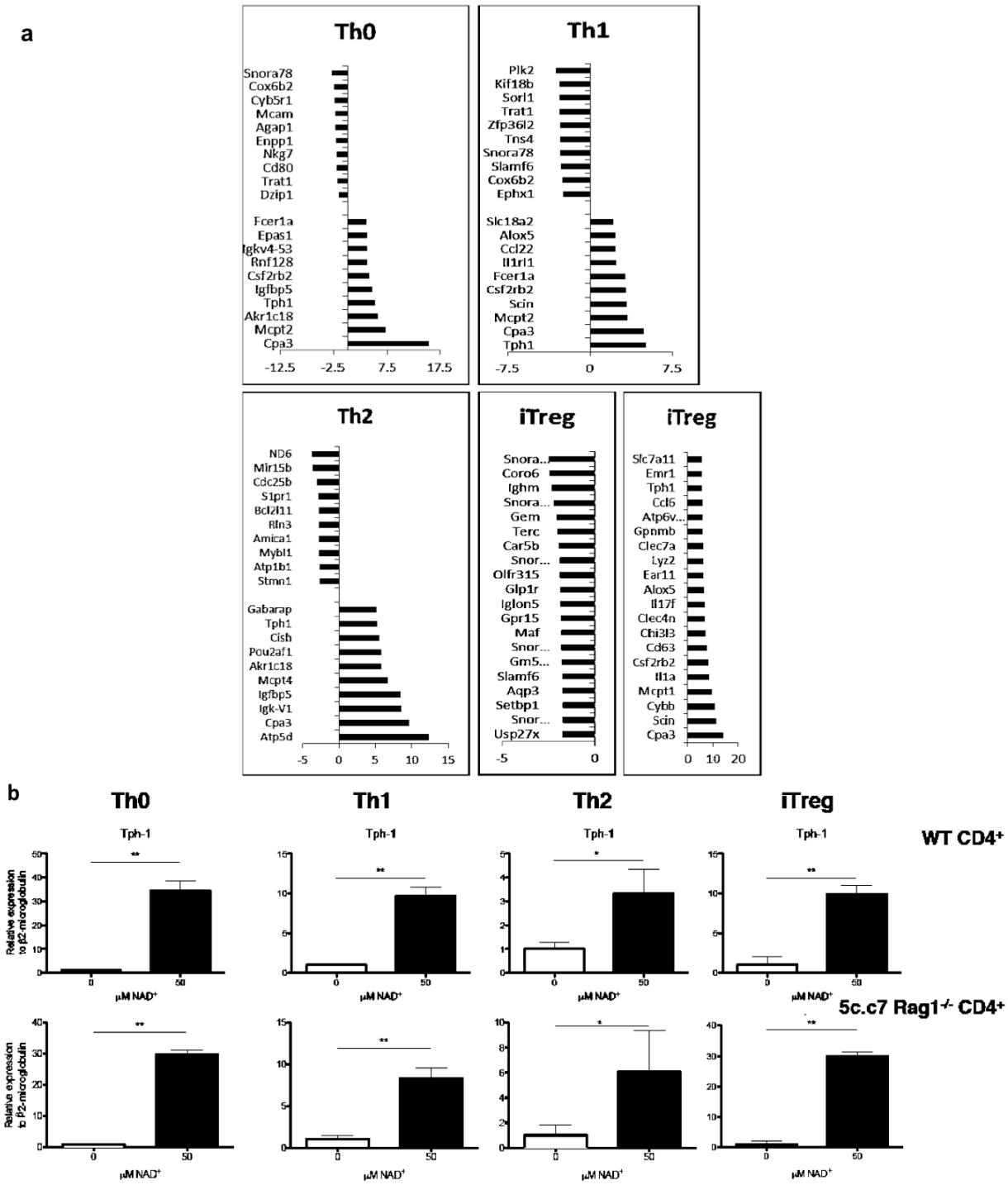
Supplementary Figure 7: NAD$^+$ does not affect Th17 differentiation. Sorted naïve CD4$^+$ T cells were isolated from spleens of 5C.C7 *Rag2*$^{-/-}$ mice (C57BL/6 background) and were activated with α-CD3/α-CD28 antibodies only or in Th17 polarizing conditions (with α-CD3/α-CD28, 4ng/ml of recombinant TGFβ and 100ng/ml of recombinant IL-6, 10μg/ml of anti-IL-4, anti-IL-12 and anti-IFNγ antibodies). Cells in Th17 polarizing conditions were cultured in presence of increasing concentrations of NAD$^+$. After 96 hours percentage of CD4$^+$ IL-17A$^+$ IFNγ^+, CD4$^+$ IL-17A$^+$ IL-4$^+$ IL-10$^+$ and CD4$^+$ IL-17A$^+$ IL-10$^+$ cells were measured by flow cytometry by gating on non-apoptotic CD4$^+$ cells. To set the gates, flow cytometry dot plots were based on comparison with isotype controls, fluorescence minus one (FMO), permeabilized and unpermeabilized unstained cells (n=15; the data derived from three different experiments). n.s., not significant. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, as determined by Student's t-test, comparing the indicated groups.



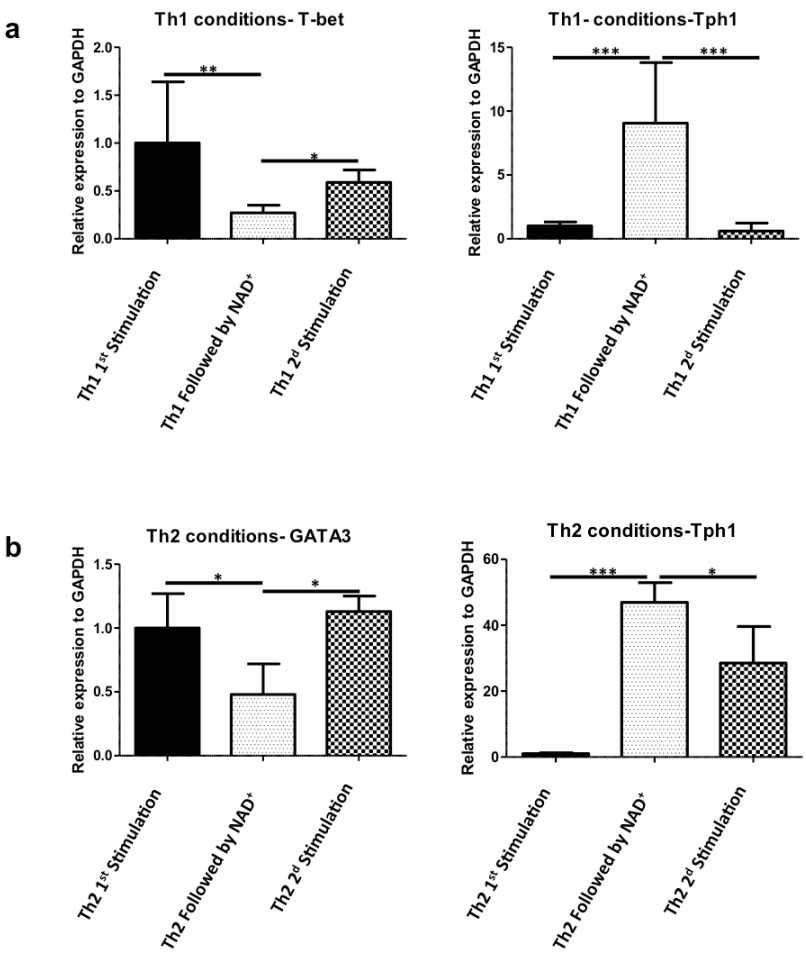
Supplementary Figure 8: NAD$^+$ promotes Th17 differentiation in iTreg polarizing conditions. Sorted naïve CD4$^+$ T cells were isolated from spleens of 5C.C7 *Rag2*$^{-/-}$ mice (C57BL/6 background) and were activated with α-CD3/α-CD28 antibodies only or in iTreg polarizing conditions (with α-CD3/α-CD28, 4ng/ml of recombinant TGFβ, 50ng/ml of recombinant IL-2 and 10μg/ml of anti-IL-4, anti-IL-6, anti-IL-12 and anti-IFNγ antibodies). Cells in iTreg polarizing conditions were cultured in presence of increasing concentrations of NAD$^+$. After 96 hours percentage of CD4$^+$ IL-10$^+$IL-17A$^+$ and CD4$^+$ IL-17A$^+$ TGFβ$^+$ cells was measured by flow cytometry by gating on non-apoptotic cells. To set the gates, flow cytometry dot plots were based on comparison with isotype controls, fluorescence minus one (FMO), permeabilized and unpermeabilized unstained cells (n=15; the data derived from three different experiments). n.s., not significant. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, as determined by Student's t-test, comparing the indicated groups.



Supplementary Figure 9: NAD$^+$ inhibits the expression of well known transcription factors. *T-bet*, *GATA3*, *STAT5a*, *STAT6*, *RORγt* and *Foxp3* expression of 5C.C7 *Rag2*$^{-/-}$ naïve CD4$^+$ T cells cultured under Th0, Th1, Th2 and iTreg polarizing conditions and in presence of NAD$^+$. After 24 hours of culture, cells were collected and mRNA was extracted. mRNA levels of *T-bet*, *GATA3*, *STAT5a*, *STAT6*, *RORγt* and *Foxp3* was determined by real-time PCR. Values are expressed as fold expression relative to the house-keeping gene *GAPDH*. (n=15; data derived from three different experiments). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; n.s., not significant, as determined by Student's t-test, comparing the indicated groups.



Supplementary Figure 10: NAD$^+$ promotes *Tph1* expression. (a) Microarray analysis of genes up-regulated or down-regulated over two fold in wild type (C57BL/6) naïve CD4$^+$ T cells under Th0, Th1, Th2 and iTreg conditions after 96 hours of culture and in presence of NAD$^+$(50μM) or PBS. Fold change in gene expression following treatment with 50 μM of NAD$^+$ was determined from normalized expression data. Top 10 or 15 differentially regulated genes are shown. (n=3 per condition). **(b)** *Tph1* expression of wild type (C57BL/6) and 5C.C7 *Rag2*$^{-/-}$ (C57BL/6 background) naïve CD4$^+$ T cells under Th0, Th1, Th2 and iTreg polarizing conditions after 96 hours of culture and in presence of NAD$^+$ (50μM) or PBS. After 96 hours, cells were collected and mRNA was extracted. mRNA levels of *Tph1* was determined by real-time PCR. Values are expressed as fold expression relative to the house-keeping gene *GAPDH* (n=10; data derived from three different experiments). *, $p < 0.05$; **, $p < 0.01$; n.s., not significant, as determined by Student's t-test, comparing the indicated groups.



Supplementary Figure 11: NAD$^+$ inhibits *T-bet* and *GATA3* expression in polarized Th1 and Th2 cells and increases *Tph1* expression. Sorted naïve CD4$^+$ T cells were isolated from spleens of wild type C57BL/6 mice and cultured (0.5x10^6 cells/well) in Th1 **(a)** (with α-CD3/α-CD28, 50ng/ml of recombinant IL-12, 50ng/ml of recombinant IL-2 cytokine and 10μg/ml of anti-IL-4) and Th2 **(b)** (with α-CD3/α-CD28, 50ng/ml of recombinant IL-4, 50ng/ml of recombinant IL-2, 10μg/ml of anti-IL-12 and anti-IFNγ antibodies) polarizing conditions for one week, washed and followed by an incubation period of 72 hours with NAD$^+$ (100μM). Cells were then rested for 72 hours, before a second Th1 **(a)** and Th2 **(b)** stimulation for 10 days. After each incubation period, cells were collected and mRNA level of *T-bet*, *GATA3* and *Tph1* was assessed by real-time PCR. Values are expressed as fold expression relative to the house-keeping gene *GAPDH* (n=10; data derived from three different experiments). n.s., not significant. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$, as determined by Student's t-test, comparing the indicated groups.

Th0

Ingenuity Canonical Pathways	*-log(p-value)*
Eicosanoid Signaling	2.56E00
VDR/RXR Activation	2.54E00
Methylglyoxal Degradation III	2.51E00
Androgen Biosynthesis	2.41E00
Crosstalk between Dendritic Cells and Natural Killer Cells	2.38E00
Communication between Innate and Adaptive Immune Cells	2.37E00
CTLA4 Signaling in Cytotoxic T Lymphocytes	2.36E00
B Cell Development	2.23E00
Retinoate Biosynthesis I	2.13E00
iCOS-iCOSL Signaling in T Helper Cells	2.12E00
Serotonin Receptor Signaling	1.98E00
Graft-versus-Host Disease Signaling	1.91E00
Autoimmune Thyroid Disease Signaling	1.87E00
Bile Acid Biosynthesis, Neutral Pathway	1.87E00
Hepatic Fibrosis / Hepatic Stellate Cell Activation	1.86E00

Th1

Ingenuity Canonical Pathways	*-log(p-value)*
Mitotic Roles of Polo-Like Kinase	3.95E00
Cell Cycle: G2/M DNA Damage Checkpoint Regulation	3.26E00
IL-10 Signaling	2.68E00
T Helper Cell Differentiation	2.66E00
IL-3 Signaling	2.63E00
FLT3 Signaling in Hematopoietic Progenitor Cells	2.58E00
Acute Myeloid Leukemia Signaling	2.53E00
Asparagine Biosynthesis I	2.43E00
IL-17A Signaling in Gastric Cells	2.41E00
NRF2-mediated Oxidative Stress Response	2.34E00
Serotonin Receptor Signaling	2.17E00
iCOS-iCOSL Signaling in T Helper Cells	2.13E00
IL-17A Signaling in Fibroblasts	2.12E00
Molecular Mechanisms of Cancer	2.07E00
April Mediated Signaling	2.05E00

Th2

Ingenuity Canonical Pathways	*-log(p-value)*
Primary Immunodeficiency Signaling	6.78E00
B Cell Development	3.94E00
FcγRIIB Signaling in B Lymphocytes	3.52E00
Hematopoiesis from Pluripotent Stem Cells	3.15E00
Remodeling of Epithelial Adherens Junctions	2.68E00
Role of NFAT in Regulation of the Immune Response	2.63E00
Phospholipase C Signaling	2.61E00
p70S6K Signaling	2.59E00
Atherosclerosis Signaling	2.58E00
PI3K Signaling in B Lymphocytes	2.45E00
Altered T Cell and B Cell Signaling in Rheumatoid Arthritis	2.32E00
Antioxidant Action of Vitamin C	2.16E00
B Cell Receptor Signaling	2.07E00
Differential Regulation of Cytokine Production in Macrophages and T Helper Cells by IL-17A and IL-17F	2.06E00
Granzyme A Signaling	2.01E00

iTreg

Ingenuity Canonical Pathways	*-log(p-value)*
Differential Regulation of Cytokine Production in Macrophages and T Helper Cells by IL-17A and IL-17F	1.13E01
Differential Regulation of Cytokine Production in Intestinal Epithelial Cells by IL-17A and IL-17F	1.02E01
Graft-versus-Host Disease Signaling	8.88E00
Altered T Cell and B Cell Signaling in Rheumatoid Arthritis	7.61E00
Communication between Innate and Adaptive Immune Cells	7.37E00
T Helper Cell Differentiation	7.29E00
LXR/RXR Activation	7.01E00
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	6.44E00
Dendritic Cell Maturation	6.42E00
Role of Cytokines in Mediating Communication between Immune Cells	5.76E00
Atherosclerosis Signaling	5.25E00
IL-10 Signaling	4.96E00
Role of NFAT in Regulation of the Immune Response	4.74E00
Role of Osteoblasts, Osteoclasts and Chondrocytes in Rheumatoid Arthritis	3.88E00
Type I Diabetes Mellitus Signaling	3.8E00

Supplementary Table 1: Ingenuity canonical pathways in naive CD4$^+$ T cells under different polarizing conditions after 96 hours of culture. Fold change in gene expression following treatment with NAD$^+$ (50μM) versus PBS was determined from normalized expression data. Genes that were differentially expressed at least 2-fold were uploaded to IPA analysis and a canonical pathway analysis was performed. Top 15 most affected pathways are shown.