Legends for Supplementary Figures

Figure S1. Identification of transcription factor binding motifs on the IL-9 promoter. (*A*) Comparative bioinformatic analysis was performed by using rVista (2.0) program that depicts % conservation between mouse (as a base) and human IL-9 loci. Boxed region indicates the IL-9 promoter. (*B*) The prediction of transcription factor binding sites was performed using TRANSFAC database analysis at a matrix similarity of 0.8.

Figure S2. Analysis of IL-9 expression in T helper cells. $CD4^+$ T cells were cultured under Th1,Th2 and Th9 skewing conditions. Relative levels of subtype specific signature cytokines (IFN-γ in Th1, IL-4 in Th2 and IL-9 in Th9, respectively) were analyzed by qRT-PCR under no stimulation or PMA/ionomycin stimulation by normalizing to the expression level of housekeeping gene HPRT (*A-C*). The levels of secreted cytokines in the culture supernatant were analyzed by ELISA (*D-F*). The data shown are expressed as mean ± SEM, n = 3 and *P<0.05, ** P<0.01, ***P<0.001.

Figure S3. Expression kinetics of IL-9 in Th9 cells. (*A-B*) Relative level of IL-9 was analyzed by qRT-PCR during *in vitro* Th9 cell differentiation (A) or at day 5 (*B*) after stimulation with PMA/ionomycin for the indicated time periods by normalizing to the expression level of housekeeping gene HPRT.

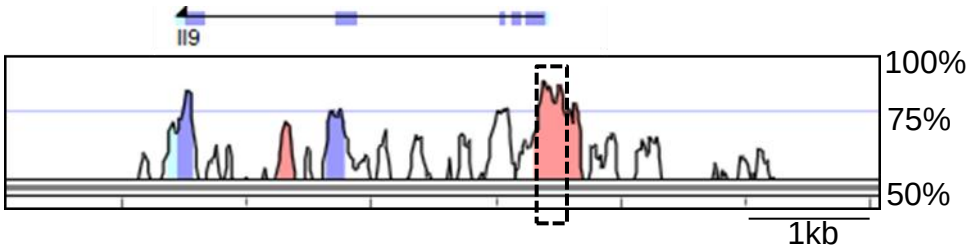
Figure S4. NFAT1 deficiency reduces IL-9 expression in $CD4^+$ T cell blasts.
$CD4^+$ T cells isolated from wild type (WT) and $NFAT1^{-/-}$ mice were restimulated with PMA/ionomycin for the indicated time periods and relative levels of IL-9 transcript were measured by qRT-PCR (*A*) and ELISA (*B*). The data shown are expressed as mean ± SEM, n = 3 and *P<0.05, ** P<0.01.

Figure S5. Effect of IFN-γ signaling on IL-9 expression. $CD4^+$ T cells from wild type (WT) and $NFAT1^{-/-}$ (KO) were differentiated under IL-4 and TGF-β cytokine milieu in the presence or absence of α-IFN-γ. On day 5, cells were restimulated with PMA/ionomycin (PI) for 24 hrs and the levels of the secreted cytokines (IL-9 and IFN-γ) in the cell culture supernatants were analyzed using ELISA. The data shown are expressed as mean ± SEM, n = 3 and *P<0.05, ** P<0.01, ***P<0.001.

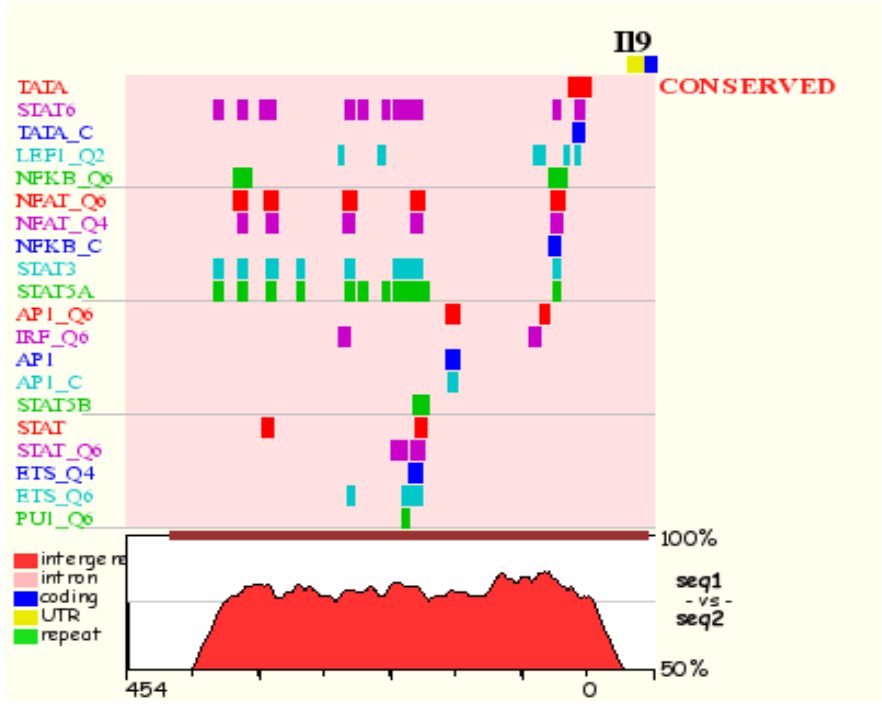
Figure S6. IL-9 promoter activity in wild type (WT) and $NFAT1^{-/-}$ (KO) cells. Th9 cells differentiated from Wild type (WT) and $NFAT1^{-/-}$(KO) mice were transfected with the empty vector (Mock), IL-9 promoter reporter construct or mutated IL-9 promoter reporter construct that has mutation in both NF-κB binding sites (mt-IL-9 Pro; NT/κB1 and NT/κB2). After 18 hrs of transfection, cells were stimulated with PMA/Ionomycin for additional 8 hrs and luciferase assay was performed. The luciferase activity was calculated relative to the activity of Renilla luciferase and represented as Relative Luciferase Unit (RLU) as a fold difference relative to the control (Mock/empty plasmid) value. The data is representative of at least 3 independent experiments. Inset shows relative levels of NFAT1, p65 and Lamin B protein in the Wild Type (WT) and $NFAT1^{-/-}$ (KO) cells.

Supplementary Figure 1

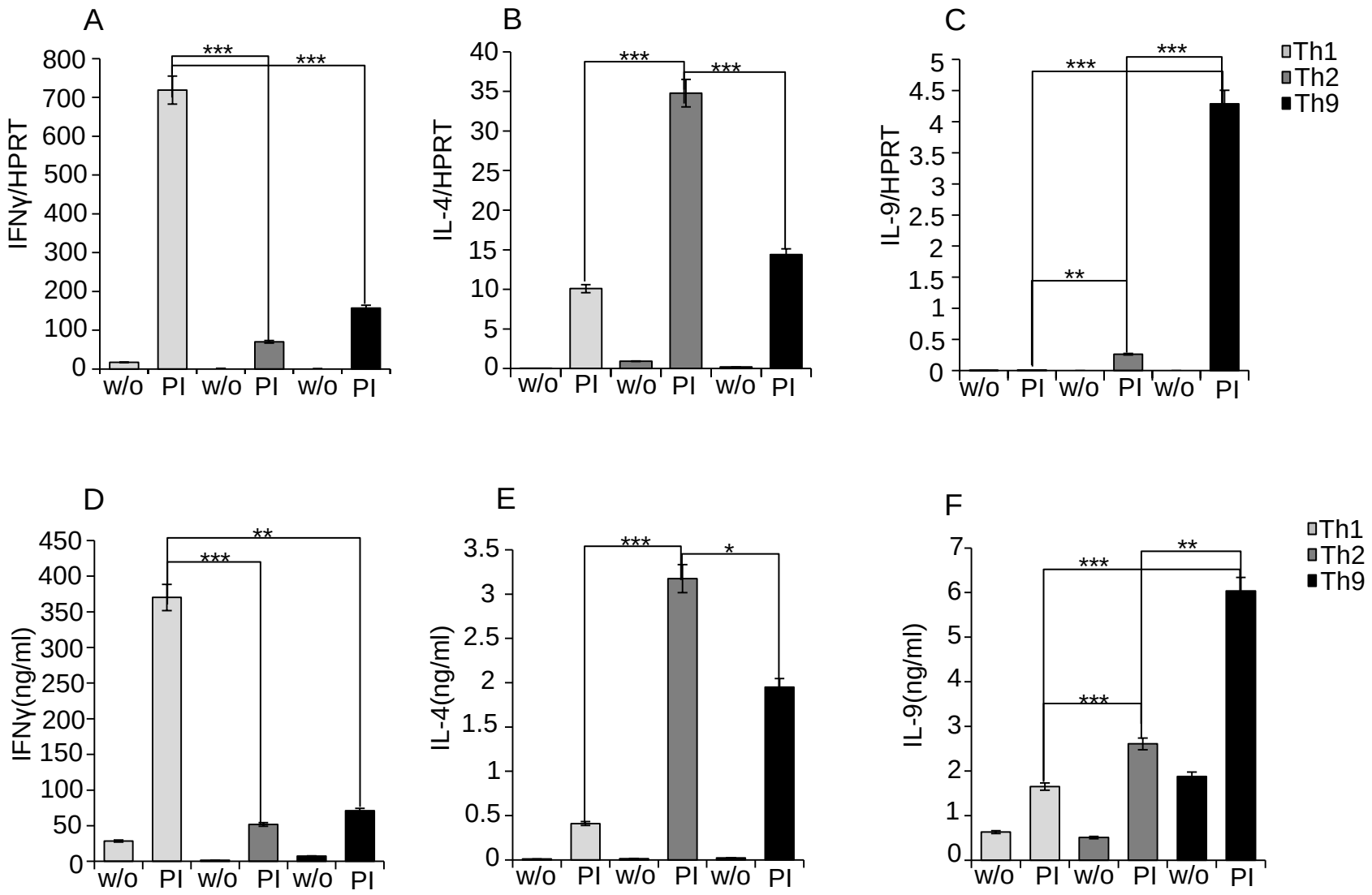
A



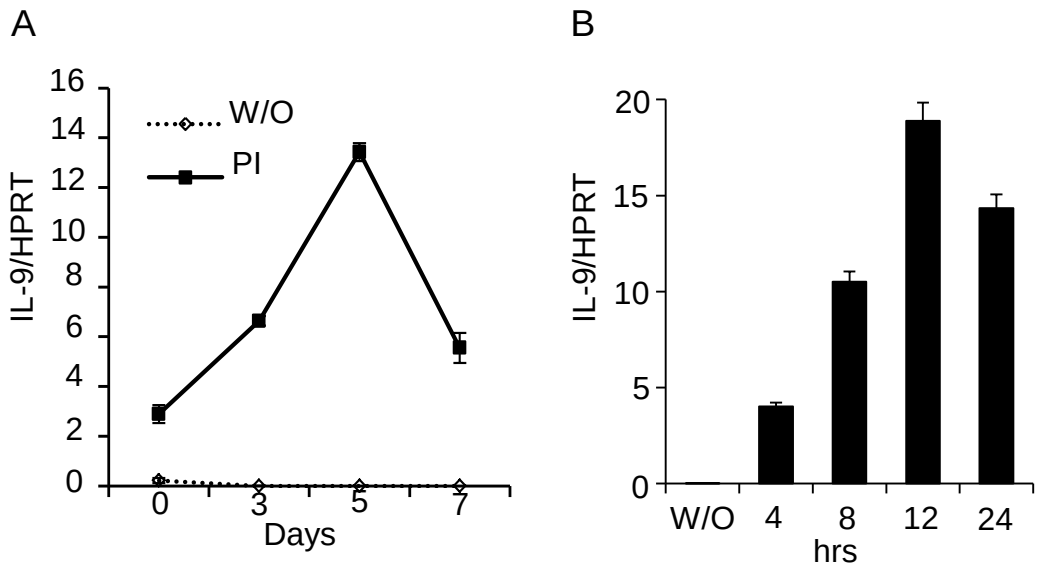
B



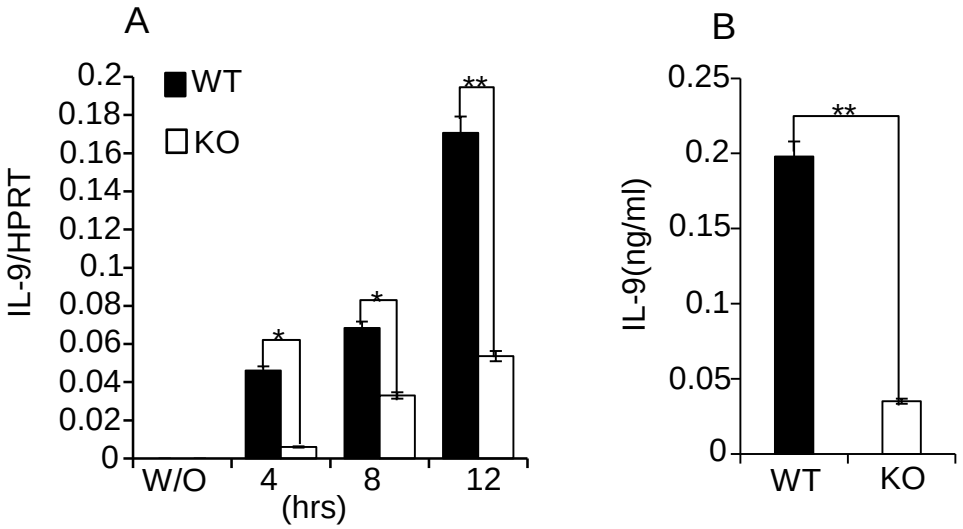
Supplementary Figure 2



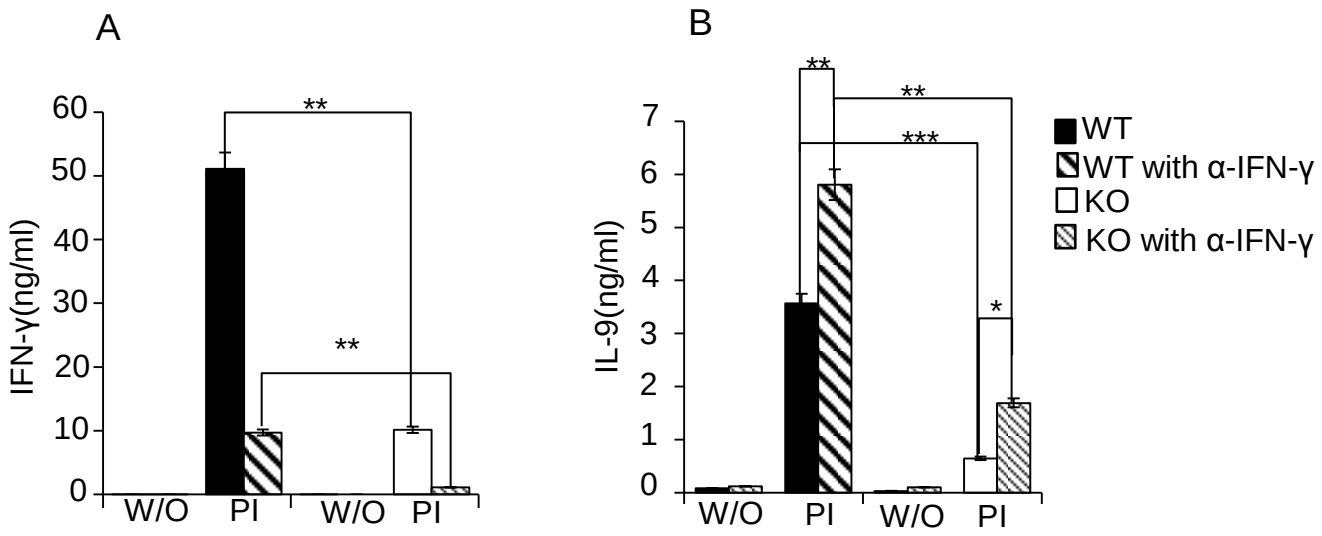
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6