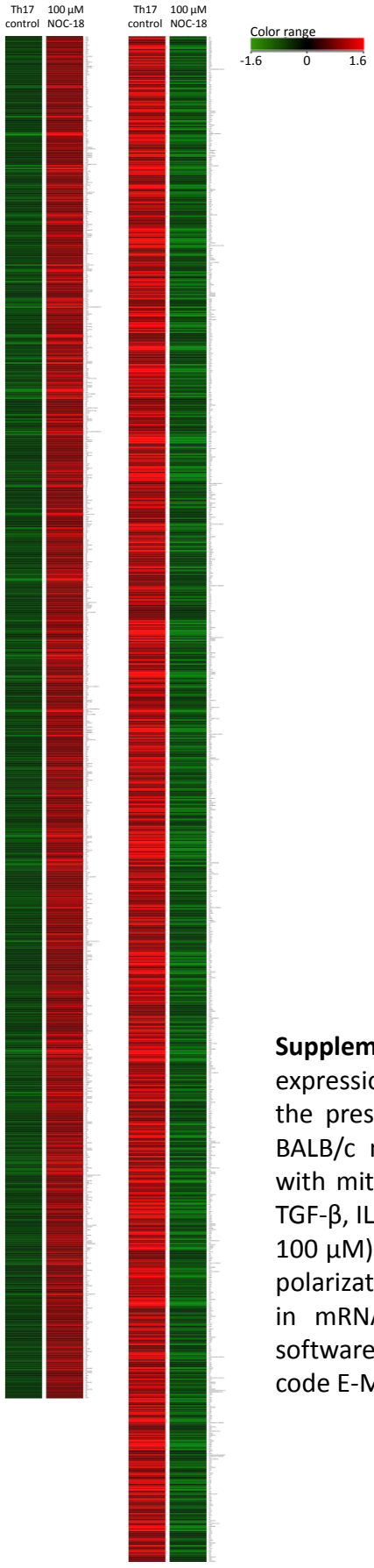
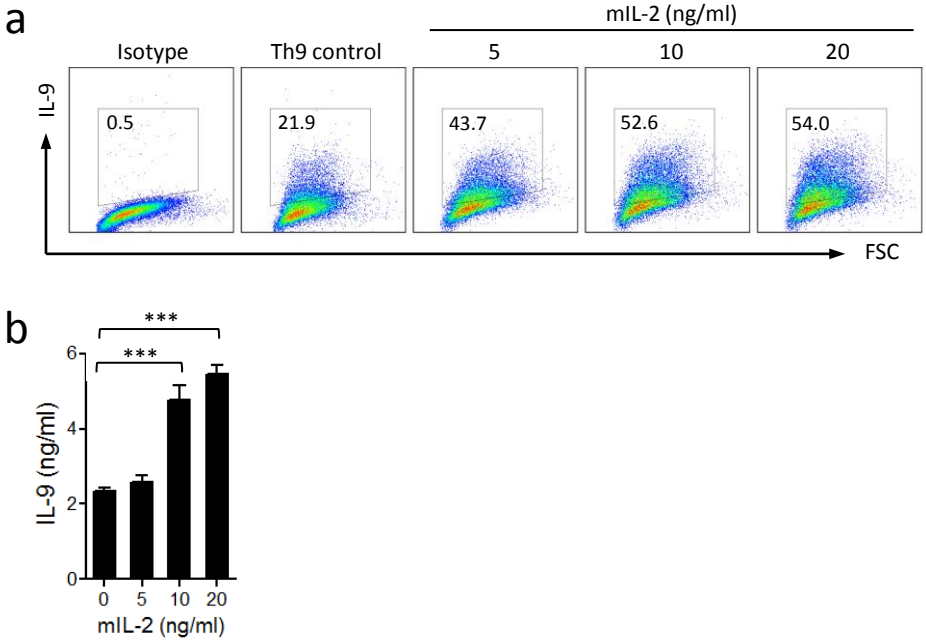
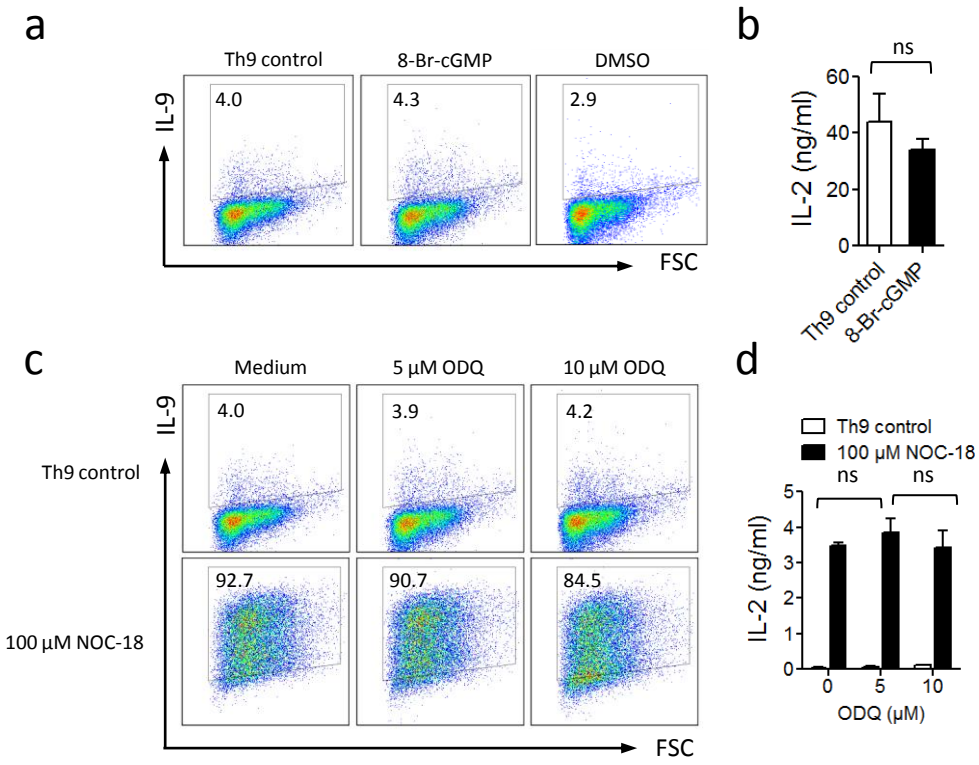


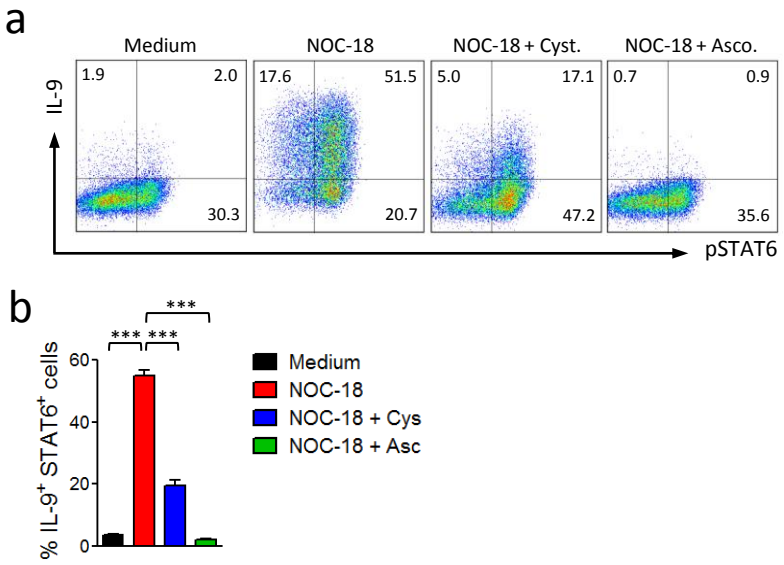
Supplementary Figure 1 Microarray analysis of gene expression in $CD4^+$ T cells under Th7 polarization conditions in the presence or absence of NO. Purified $CD4^+$ T cells from BALB/c mice were cultured in round-bottom 96-well plate with mitomycin-C-treated APC and anti-CD3, anti-CD28, IL-6, TGF-β, IL-23, IL-1β, anti-IFNγ and anti-IL-4. NO donor (NOC-18, 100 µM) was added at the start of the culture. After 3 days of polarization, cells were harvested and the relative alterations in mRNA were analysed using the Microarray Suite 5.0 software (Affymetrix) and deposited under the accession code E-MEXP-3959.



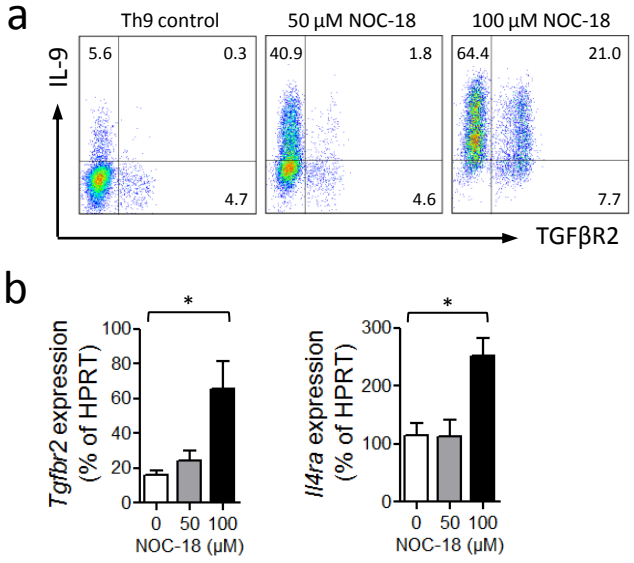
Supplementary Figure 2 IL-2 enhances Th9 differentiation. $CD4^+$ T cells from BALB/c mice were polarized under Th9 polarizing conditions in the presence of IL-2. (**a**) Percentage $IL\text{-}9^+$ T cells on day 4 were analyzed by FACS and (**b**) IL-9 concentration in the culture supernatant on day 5 determined by ELISA. Data are representative of 3 experiments; mean ± SEM, n=3, ***P<0.001



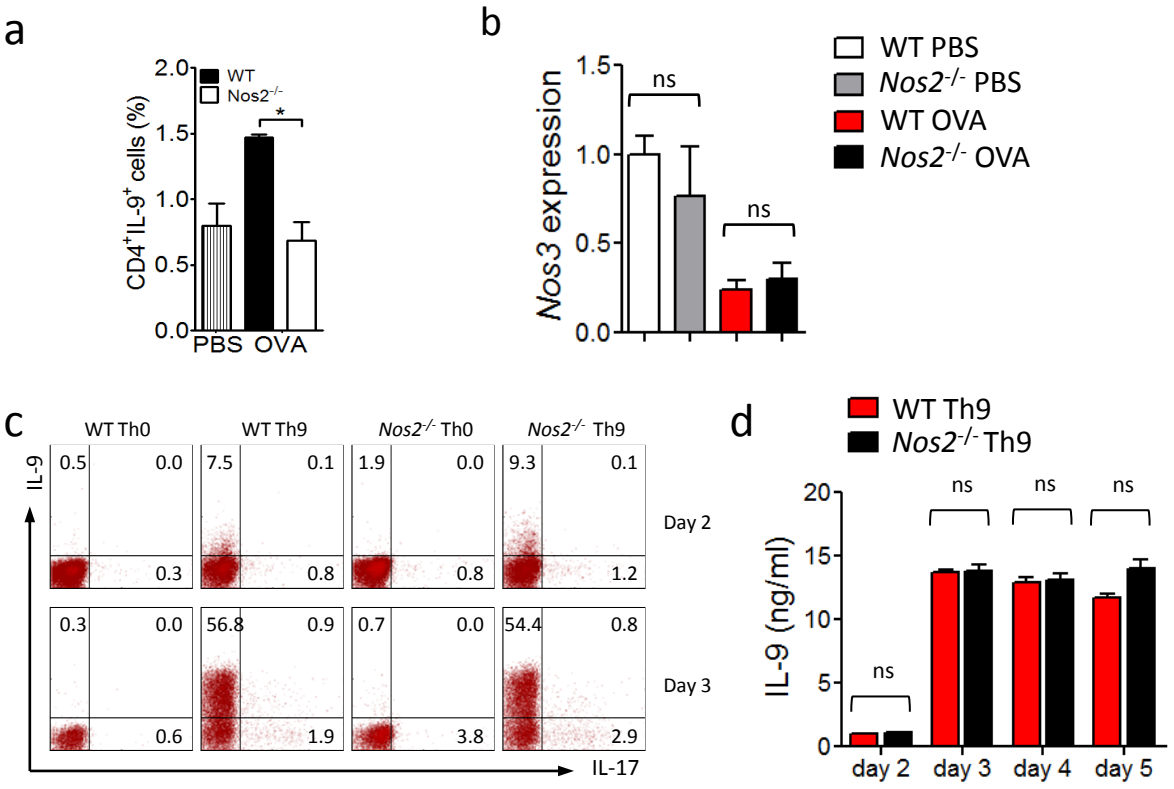
Supplementary Figure 3 NO-mediated enhancement of Th9 differentiation is independent of cGMP. $CD4^+$ T cells from BALB/c mice were cultured under Th9 polarizing conditions with 8-Br-cGMP (5 mM) or the vehicle (DMSO). (**a**) Percentage of IL-9^+ T cells was analyzed by FACS on day 4. (**b**) IL-2 in the culture supernatant on day 5 determined by ELISA. $CD4^+$ T cells were polarized under Th9 conditions ± NO in the presence of ODQ, a specific inhibitor of cyclic guanylate cyclase. (**c**) Percentage IL-9^+ T cells on day 4 were analyzed by FACS and (**d**) IL-2 concentration in the culture supernatant on day 5 determined by ELISA. Data are representative of 3 experiments; mean ± SEM, n=3, ns = not significant.



Supplementary Figure 4 NO induces STAT6 phosphorylation during Th9 cell differentiation. $CD4^+$ cells were polarized under Th9 polarizing conditions in the presence of NOC-18 (100 μM) and cysteine (1 mM) or ascorbate (300 μM). (**a**) Percentage of $IL\text{-}9^+pSTAT6^+$ T cells on day 5 were analyzed by FACS. (**b**) Pooled results of 3 experiments. Data are mean ± SEM, n=3, ***P<0.001.



Supplementary Figure 5 TGFβR1 and IL-4R expression are up-regulated by NO during Th9 differentiation. CD4 T cells from BALB/c mice were cultured under Th9 polarizing conditions with NOC-18 (100 µM). (**a**) Percentage of IL-9^{+} and TGFβR2^{+} T cells on day 3 analyzed by FACS. (**b**) *Tgfβr2* and *Il4rα* mRNA expression on day 3 was determined by qPCR. Data are representative of 3 independent experiments; mean ± SEM, n=3, * P<0.05.



Supplementary Fig. 6. (**a**) WT and *Nos2*$^{-/-}$ mice were immunized s.c. with OVA on day 1 and 7 and sacrificed on day 10. Draining LN cells were harvested and analyzed for CD4^{+}IL-9^{+} cells by FACS. Hatched column = pooled of DLN of 3 naïve WT and 3 *Nos2*$^{-/-}$ mice which had similar % of Th9 cells. Data are representative of 2 independent experiments; mean ± SEM, n=4, * P<0.05.
(**b**) qPCR analysis of *Nos3* expression in the lung of WT and *Nos2*$^{-/-}$ mice immunized s.c. with OVA (days 1 and 7) and challenged i.n. (days 14-16) with OVA or PBS. Lung cells were harvested on day 17. Vertical bars are mean ± SEM, n=5, ns = not significant. (**c**) Percentage of Th9 cells (FACS, representative of 5 mice per group) and IL-9 production (ELISA in culture supernatant) by CD4^{+} T cells from *Nos2*$^{-/-}$ and WT mice polarized *in vitro* for 3 days under Th9 differentiation conditions (plate-bound αCD3, αCD28, IL-4, TGFβ, IL-2). ELISA, mean ± SEM, n=5.