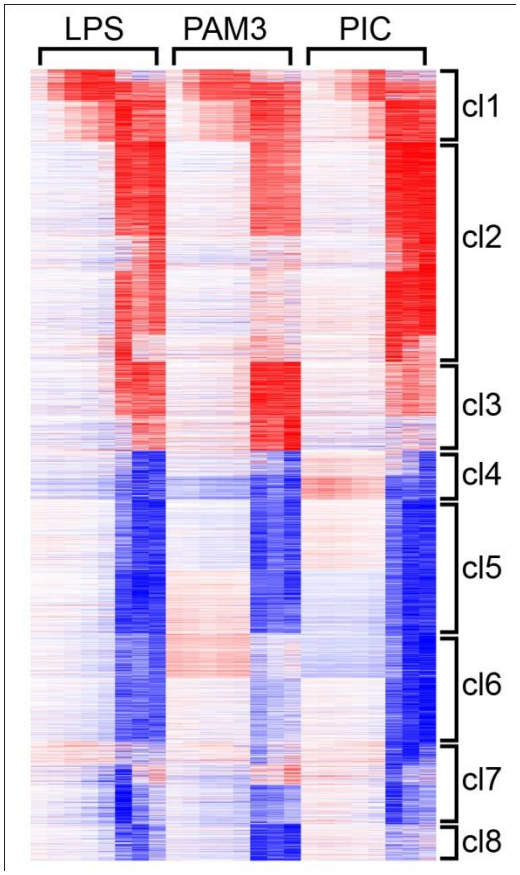
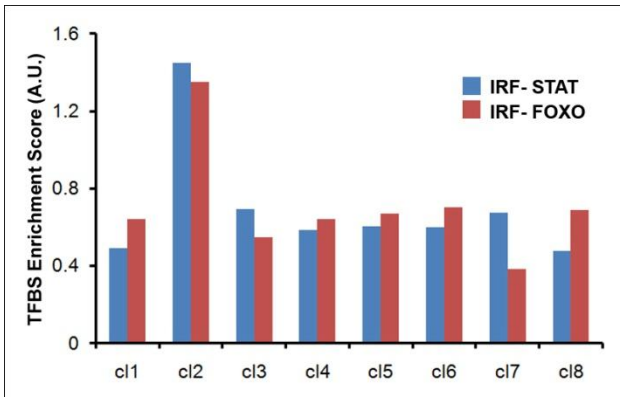
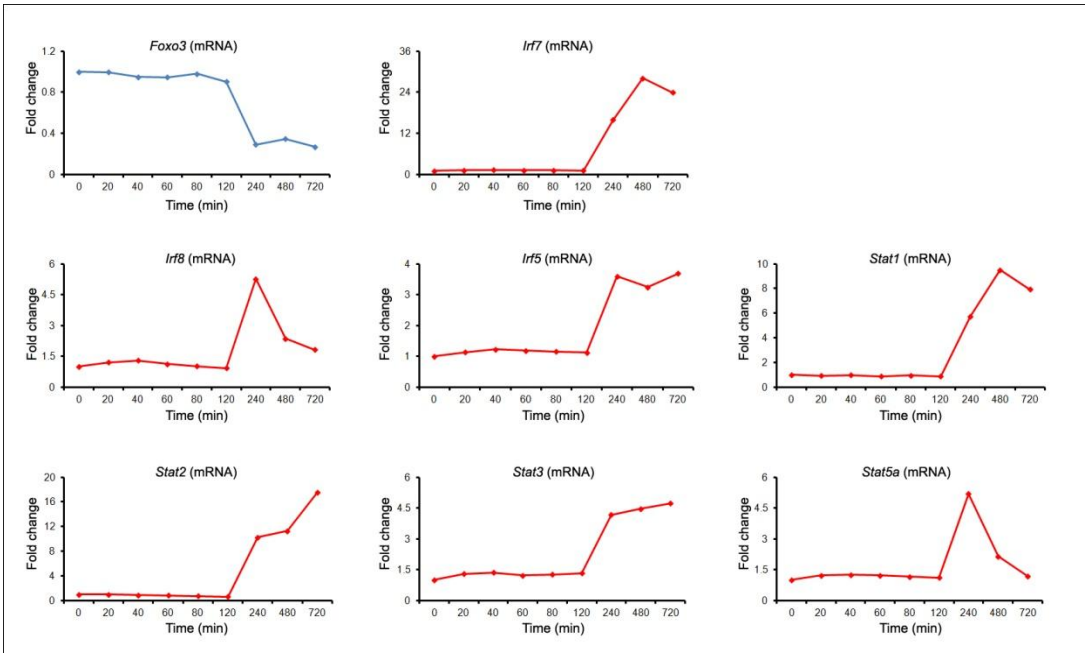


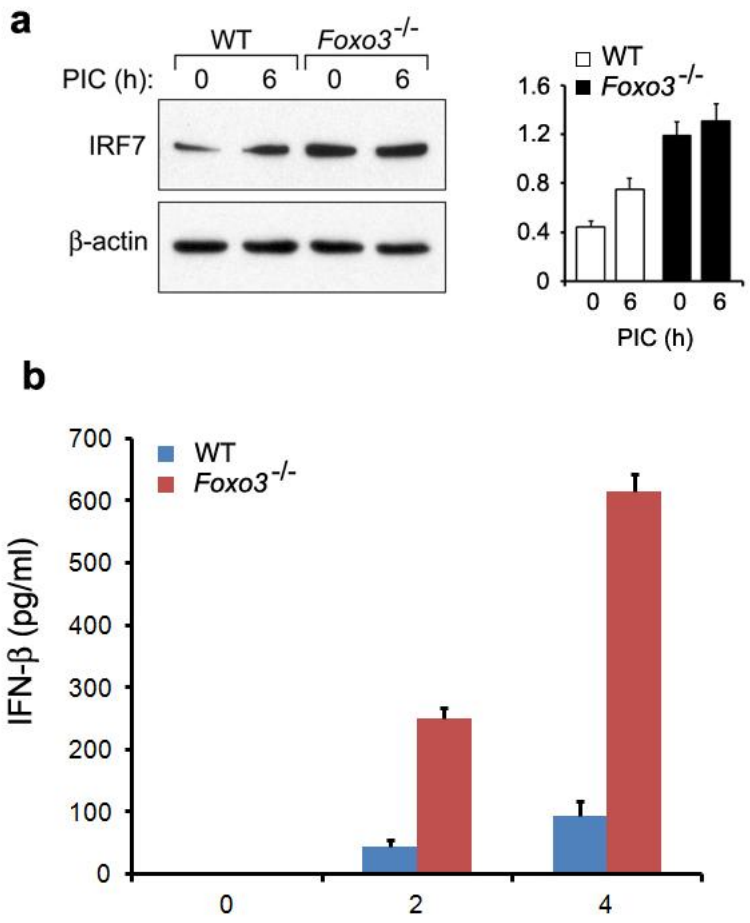
Supplementary Figure 1. Gene expression analysis in macrophages. Microarray analysis of mRNA from wild-type BMMs stimulated for 20, 40, 60, 80, 120, 240, 360 and 480 minutes with LPS (10ng/ml), Pam3 (300ng/ml) or PIC (6μg/ml) is presented as a heat map. Gene cluster analysis was performed using the K-means algorithm with squared Euclidean distance and clearly distinguished gene clusters induced or repressed by specific TLR agonists. Relative expression values are color-coded (red: TLR-induced, blue: TLR-repressed). Data represent the average of three independent experiments.



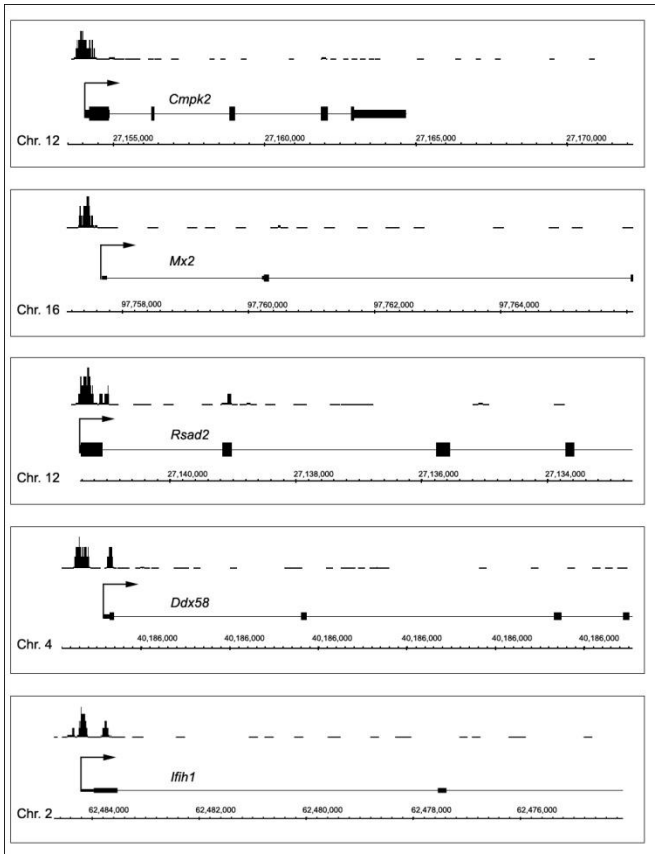
Supplementary Figure 2. Motif scanning analysis identifies overrepresentation of IRF-STAT and IRF-FOXO TFBS pairs in cis-regulatory regions of cluster 2 genes. Shown are relative IRF-STAT and IRF-FOXO TFBS pair-wise enrichment scores per gene cluster. This score indicates the relative frequency of co-occurrence of TFBS pair within a 250bp proximity limit to each other in a gene promoter region. Overrepresentation of IRF-STAT and IRF-FOXO TFBS pairs in the promoter regions of cluster 2 genes provides an indication for transcriptional co-regulation of IRF, STAT and FOXO transcription factors.



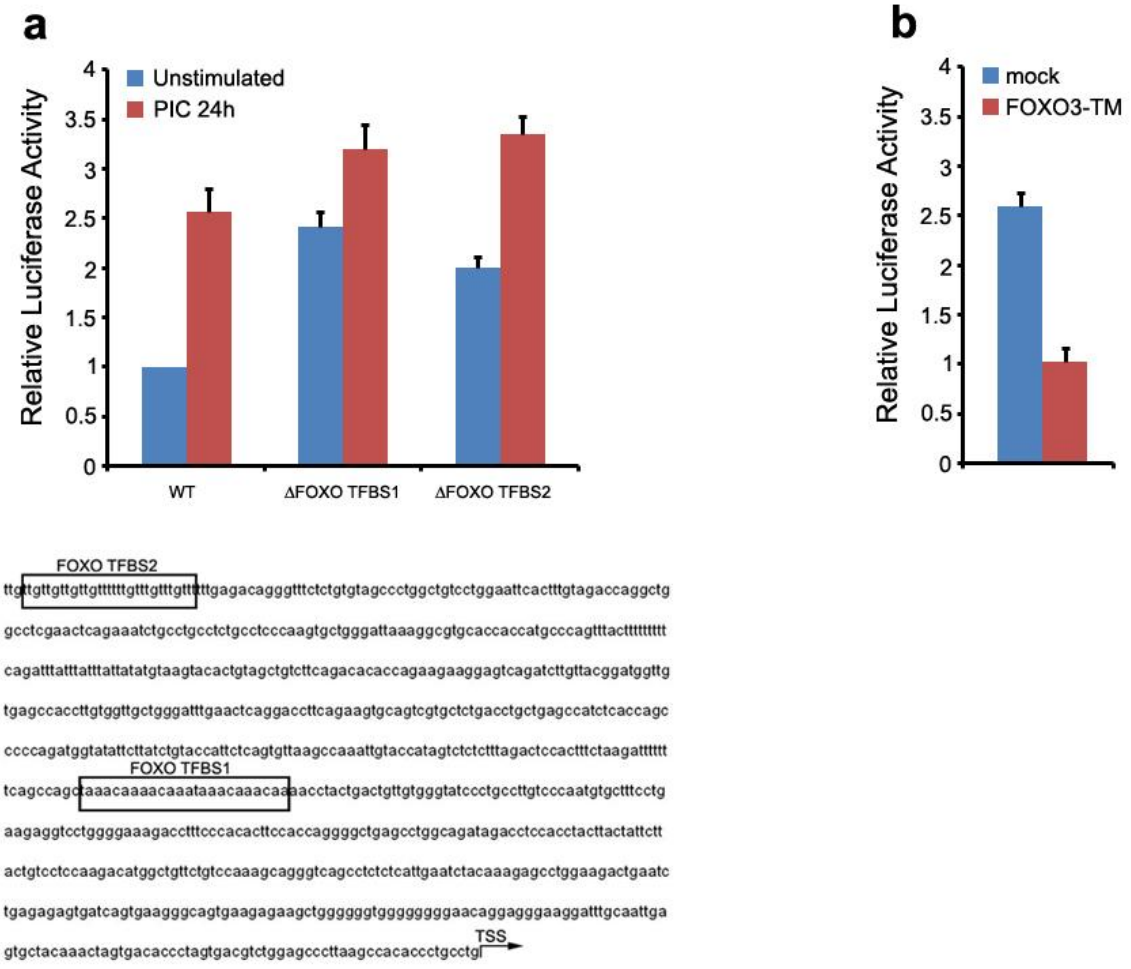
Supplementary Figure 3. Transcriptional profiling of PIC-induced and PIC-repressed genes encoding for transcription factors. Shown are mRNA levels of genes encoding for *Irf5*, *Irf7*, *Irf8*, *Stat1*, S*tat2*, *Stat3*, *Stat5a* and *Foxo3* in wild-type BMMs stimulated for 60, 120, 240, and 480 minutes with PIC (6μg/ml) normalized to that in unstimulated cells.



Supplementary Figure 4. Increased IRF7 and IFNβ protein levels in *Foxo3*-null macrophages. a, Immunoblot of IRF7 demonstrates that PIC-stimulation of wild type macrophages results in a significant increase in IRF7 protein levels. Increased protein levels of IRF7 were observed in Foxo3 null BMMs under basal conditions. Bar graph demonstrates densitometric quantification of IRF7 protein levels. The data represent the average of three independent experiments ± standard error. **b,** Enzyme-linked immunosorbent assay (ELISA) of IFNβ in supernatants of WT and *Foxo3*-null BMMs stimulated with PIC (6μg/ml) for the indicated times. The data represent the average of three independent experiments ± standard error.

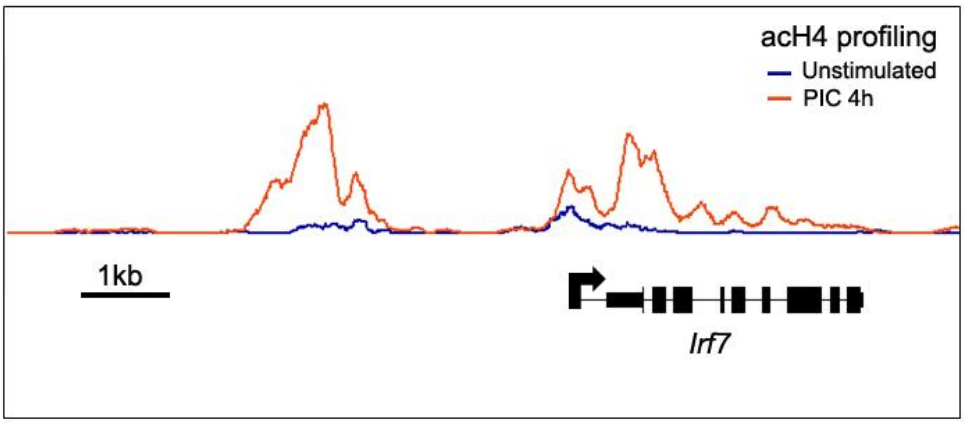


Supplementary Figure 5. Direct targets of FOXO3. ChIP-Seq analysis demonstrates FOXO3 binding profiles at *Cmpk2, Mx2, Rsad2, Ddx58 and Ifih1* gene promoters in wild type unstimulated BMMs. The ChIP-Seq data are aligned to the mouse genome (NCBI37/mm9; July 2007). The arrow represents transcriptional start site of a gene. ChIP-Seq data are shown in reads per million. Data are representative of two experiments.

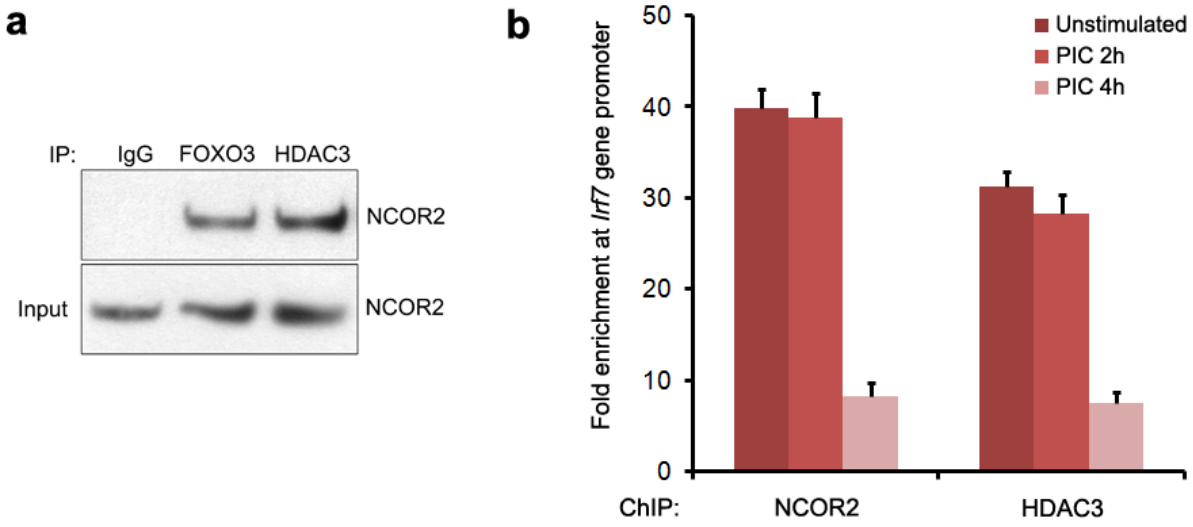


Supplementary Figure 6. FOXO3 represses *Irf7* promoter activity a, Deletion of FOXO TFBS in the *Irf7* gene promoter resulted in an increased basal *Irf7* promoter activity. RAW 264.7 cells were transiently transfected with *Irf7* promoter luciferase reporter constructs including wild-type *Irf7* promoter and FOXO TFBS deletion mutants of *Irf7* promoter, as indicated in the lower panel. The data represent the average of three independent experiments ± standard error. The results were expressed as fold induction over the activity of wild type *Irf7* promoter luciferase reporter in unstimulated cells. **b,** RAW 264.7 cells were transiently co-transfected with

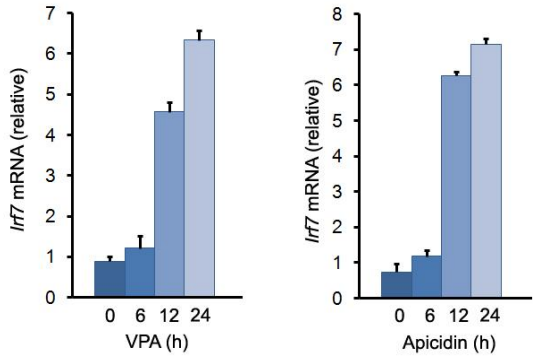
Irf7 promoter luciferase reporter construct and constitutively active FOXO3 (FOXO3-TM). Cells were stimulated with PIC for 24 hours. All luciferase activity was normalized to the expression of the cotransfected Renilla luciferase. The data represent the average of three independent experiments ± standard error.



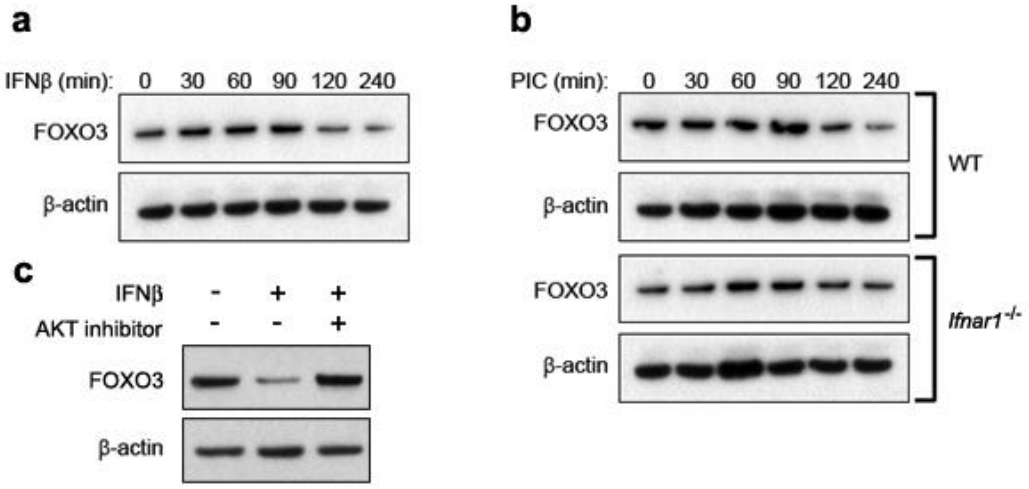
Supplementary Figure 7. Enhanced histone acetylation at *Irf7* gene promoter in activated macrophages. Shown are ChIP-Seq profiles of acetylated histone H4 in wild type BMMs in the presence (red line) or absence (blue line) of PIC. The arrow represents transcriptional start site of a gene. ChIP-Seq data are shown in reads per million. Data are representative of two experiments.



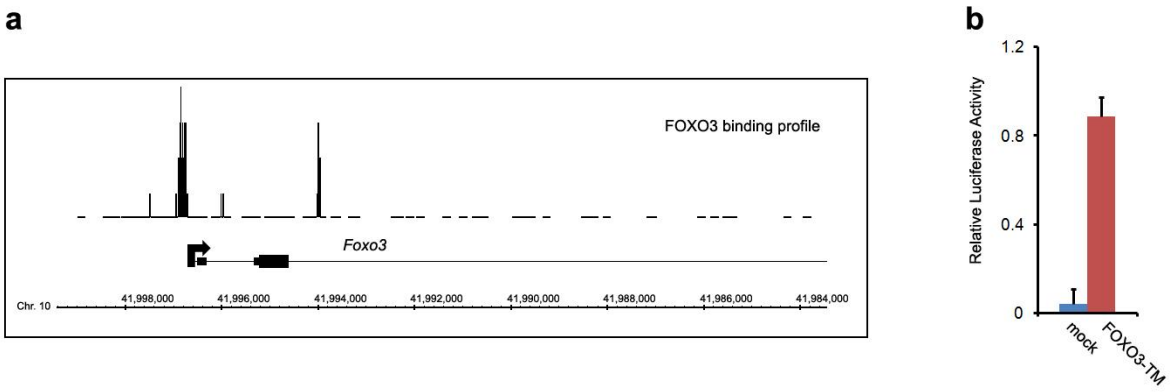
Supplementary Figure 8. FOXO3 interacts with NCOR2 and HDAC3 a, Co-immunoprecipitation analysis demonstrates interaction of FOXO3 with nuclear co-repressor 2 (NCOR2) and histone deacetylase 3 (HDAC3) in macrophages. Unstimulated macrophages from wild-type mice were lysed and processed for immunoprecipitation with either anti-FOXO3 or anti-HDAC3 antibodies and immunoblotted with anti-NCOR2. Immunoprecipitation with IgG was used as a negative control. Data are representative of two independent experiments. **b,** ChIP analysis of NCOR2 and HDAC3 binding at *Irf7* gene promoter. Stimulation with PIC (6μg/ml) results in the clearance of NCOR2 and HDAC3 from *Irf7* gene promoter. Data was normalized to IgG (negative control) and represent the average of three independent experiments ± standard error.



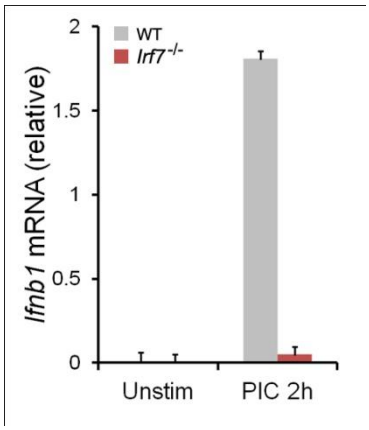
Supplementary Figure 9. Treatment with HDAC inhibitors increases *Irf7* mRNA levels in macrophages. Wild type BMMs were treated with Valproic acid (VPA, 5mM), and Apicidin (2.5µM), for the indicated times and *Irf7* mRNA levels were measured by quantitative real-time RT–PCR analysis. Data represent the average of three independent experimental values ± standard error.



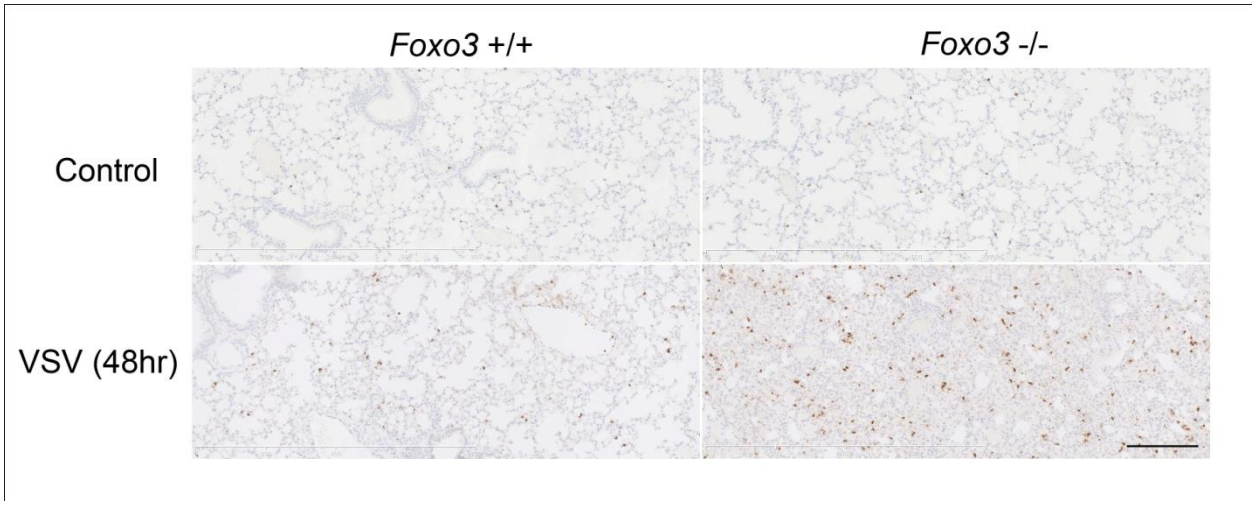
Supplementary Figure 10. IFN represses FOXO3 protein levels. Immunoblot of FOXO3 demonstrates that **(a)** Ifnβ- or **(b)** PIC-stimulation of wild type macrophages results in a significant decrease in FOXO3 protein levels. **b,** PIC-induced decrease of FOXO3 protein levels was not observed in *Ifnar1*-null cells. **c,** Treatment of the cells with AKT inhibitor IV abrogated Ifnβ-induced decrease in the FOXO3 protein level. Data are representative of three experiments.



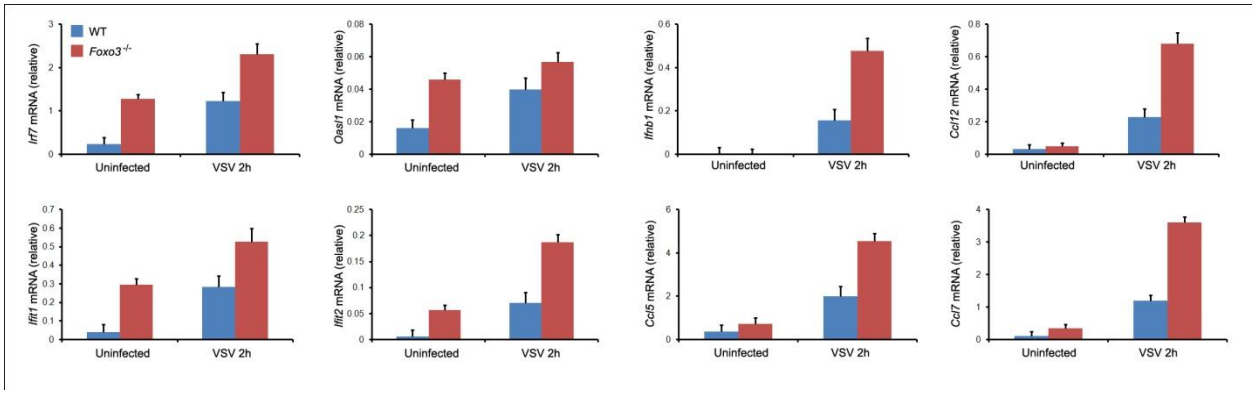
Supplementary Figure 11. Foxo3 is required for its own transcription a, ChIP-Seq analysis demonstrates FOXO3 binding profile at *Foxo3* gene promoter in wild type unstimulated BMMs. The ChIP-Seq data are aligned to the mouse genome (NCBI37/mm9; July 2007). The arrow represents transcriptional start site of a gene. ChIP-Seq data are shown in reads per million. Data are representative of two experiments. **b,** The 5'-flanking region DNA (-2500 to +500) of *Foxo3* gene was inserted into the firefly luciferase reporter. RAW 264.7 cells were transiently co-transfected with *Foxo3* promoter luciferase reporter and constitutively active FOXO3 (FOXO3-TM). All luciferase activity was normalized to the expression of the co-transfected Renilla luciferase. The data represent the average of three independent experiments ± standard error.



Supplementary Figure 12. IRF7 is a critical regulator of PIC-induced *Ifnb1* transcription in macrophages. Shown are *Ifnb1* mRNA levels in wild-type and *Irf7*$^{-/-}$ macrophages in the presence or absence of PIC stimulation. Data represent the average of three independent experiments.



Supplementary Figure 13. Enhanced neutrophil influx in the lungs of VSV-infected *Foxo3*-null mice. Immunohistochemical analysis for LY6B demonstrated elevated numbers of neutrophils in the lungs of *Foxo3*-null mice on day 2 following VSV infection, relative to WT. Data are from one experiment out of three (n=6 mice per group). Scale bar, 200μm.



Supplementary Figure 14. Enhanced antiviral response in alveolar macrophages isolated from VSV-infected *Foxo3*-null mice. Alveolar macrophages were isolated from uninfected and VSV infected WT and *Foxo3*-null mice. mRNA levels of *Irf7*, *Ifnb1*, *Oasl1*, *Ifit1*, *Ifit2*, *Ccl5*, *Ccl12* and *Ccl7* were measured by quantitative real-time RT–PCR analysis. Data represent the average of three independent experimental values ± standard error.

Supplementary Table 1. Microarray profiling in macrophages. Microarray analysis of mRNA from wild-type BMMs stimulated for 20, 40, 60, 80, 120, 240, 360 and 480 minutes with LPS (10ng/ml), Pam3 (300ng/ml) or PIC (6μg/ml) identifies 8 gene clusters.

Supplementary Table 2. Overrepresented TFBS pairs for cluster 2 genes. Motif scanning analysis identifies overrepresentation of TFBS pairs in cis-regulatory regions of cluster 2 genes. Shown are normalized TFBS pair-wise enrichment scores for cluster 2 genes.

Supplementary Table 3. Gene expression profiling of forkhead family members in PIC-stimulated macrophages. Shown are mRNA levels of genes encoding for forkhead family transcription factors in wild type macrophages either in presence or absence of PIC.

Supplementary Table 4. IFN signature genes are up-regulated in Foxo3-null BMMs under basal conditions. Shown is the output of gene set enrichment analysis of genes differentially expressed in WT and Foxo3-null macrophages under basal conditions. IFN signature genes are ranked by metric score according to their differential expression between WT and Foxo3-null macrophages.

Supplementary Table 5. IFN signature genes are up-regulated in Foxo3-null PIC-stimulated BMMs. Shown is the output of gene set enrichment analysis of genes differentially expressed in WT and Foxo3-null macrophages following PIC stimulation. IFN signature genes are ranked by metric score according to their differential expression between WT and Foxo3-null macrophages.

Supplementary Table 6. Foxo3 targets: List of 45 FOXO3 targets that were identified by combined microarray and ChIP-Seq analysis. Genes are ranked by ChIP-Seq peak score that reflects the relative abundance of FOXO3 at the target gene promoter.

Supplementary Table 7. Motif scanning of the Irf7 gene promoter. Promoter sequences encompassing 3kb on either side of the transcriptional start site of a gene were scanned using 390 murine transcription factor matrices obtained from the TRANSFAC database and the software tool MotifLocator.

Supplementary Table 8. Custom gene sets: List of microarray-derived 24 custom gene sets that were used for gene set enrichment analysis.

Supplementary Table 9. Quantitative RT-PCR primers. Taqman primers used for cDNA expression analysis of selected genes by real-time PCR.

Supplementary Table 10. ChIP primers. Sequences of oligonucleotides used for quantitative ChIP analysis of selected genes by real-time PCR.