Supplemental Figures:

ROS-Mediated Amplification of AKT/mTOR Signaling Pathway Leads to Myeloproliferative Syndrome in Foxo3$^{-/-}$ Mice

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Supplemental Figure 1

Increased Extramedullary Hematopoiesis of Foxo3$^{-/-}$ Mice is Concomitant with Hypocellular Bone Marrow. Quantification of lineage frequency in wild type and Foxo3$^{-/-}$ spleens (A) and bone marrow (B). Student's t-Test. The analyses are from at least four independent experiments.

Supplemental Figure 2

Increased Extramedullary Hematopoiesis in the Liver of Foxo3-deficient Mice. Representative histological hematoxylin and eosin staining of liver sections from Foxo3$^{+/+}$ and Foxo3$^{-/-}$ mice. Increased extramedullary myeloid hematopoiesis is marked in the liver of Foxo3$^{-/-}$ mice. Two to three mice in each group were analyzed in two independent experiments.

Supplemental Figure 3

Increased Progenitors in Foxo3$^{-/-}$ Hematopoietic Organs. Distribution and size of hematopoietic progenitors from blood. The estimated number of cells in each colony was counted. One representative of three independent experiments is shown.

Supplemental Figure 4

Reduced Expression of Anti-Oxidant Enzymes in Foxo3-deficient Primitive Hematopoietic Cells. QRT-PCR gene expression analysis of anti-oxidant enzymes in wild type and Foxo3$^{-/-}$ bone marrow lineage negative cells. Quantification of the target genes is relative to the endogenous ß-actin transcript level. Results shown as mean ± SEM of duplicate analysis of at least 3 cDNAs each generated from pool of two to three wild type and Foxo3$^{-/-}$ mice.

Supplemental Figure 5

Enhanced ROS Concentration in Highly Enriched Common Myeloid Progenitor Cell Compartment. ROS-hi concentrations were measured in common myeloid progenitor (CMP) Lin^{-} IL-7^{-} Sca-1^{-} c-kit^{+} FcgR^{low} CD34^{+} bone marrow compartment freshly isolated from wild type and Foxo3$^{-/-}$ mice (n=6). One of two independent experiments is shown.

Supplemental Figure 6

***In vivo* NAC Treatment in Wild Type and Foxo3-null Mice.** Quantification of total number of myeloid (Mac1/Gr1 positive cells) and B cells (B220 positive cells) in wild type and Foxo3$^{-/-}$ mice, 15 days after daily *in vivo* NAC (100mg/kg) or PBS treatment.

Supplemental Figure 7

Increased Activation of AKT/mTOR Signaling Pathway in Foxo3$^{-/-}$ Primitive Hematopoietic Progenitor Cell Compartment is Regulated by the Relative Loss of Lnk. Representative FACS histograms of phosphorylated AKT, mTOR and S6 ribosomal protein in mononuclear bone marrow cells derived from 5-FU-treated wild type or Foxo3$^{-/-}$ mice and retrovirally transduced with MIG-Lnk or with MIG vector control. Cells were harvested 48 hr after transduction and starved in IMDM 0.1% FCS *in vitro* for 2 hours. Intracellular pSer473AKT (upper left panel), pSer2448 mTOR (upper right panel) and pSer235/236 S6 (lower panel) were measured in GFP positive cells stimulated or not with IL-3.

Supplemental Figure 8.

ROS-dependent Regulation of Lnk mRNA Expression. QRT-PCR expression analysis of Lnk was performed in total bone marrow cells isolated from wild type mice and treated *in vitro* with or without the indicated concentration of H_2O_2 for 6 hours (n=2).

Supplemental Figure 9.

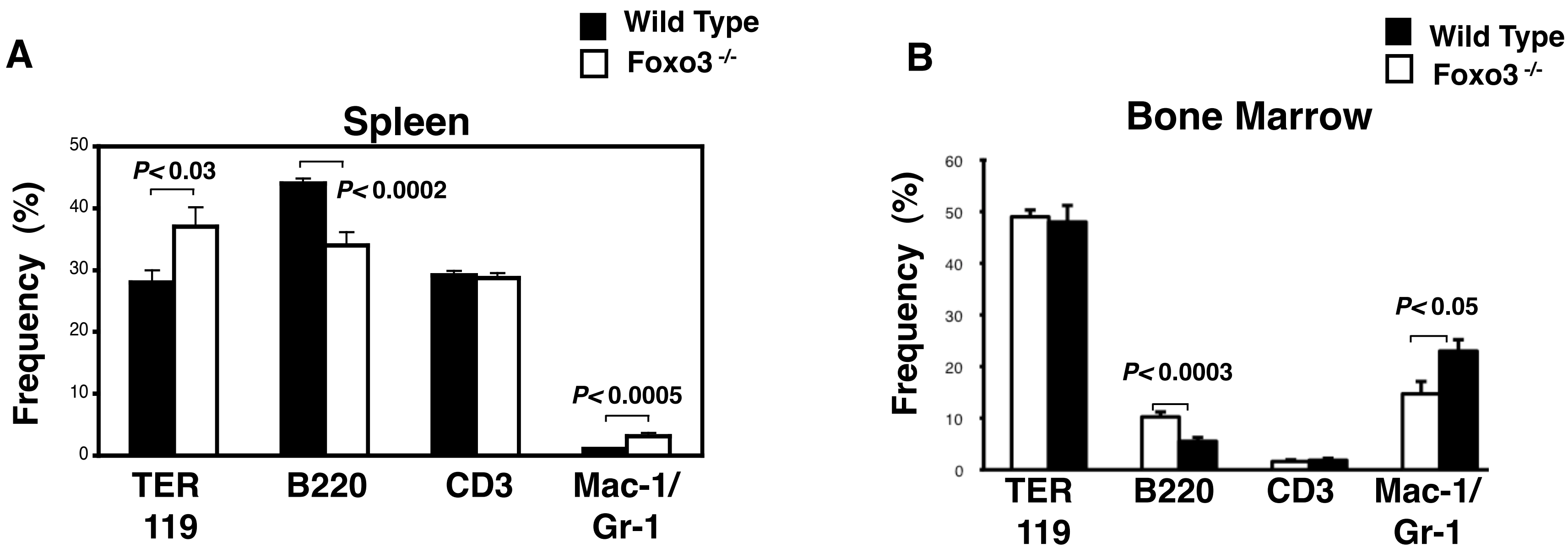
Impact of Rapamycin on ROS in Primitive Myeloid Progenitor Cells.

Endogenous ROS concentrations were measured in freshly isolated Lin^- $IL7R\alpha^-$ $Sca\text{-}1^-$ $c\text{-}Kit^+$ cells from wild type or $Foxo3^{-/-}$ mice treated *in vivo* five days a week for 12 days with rapamycin (4mg/kg) or vehicle alone; results are shown as fold change in MFI of ROS-hi in $Foxo3^{-/-}$ Lin^- $IL7R\alpha^-$ $Sca\text{-}1^-$ $c\text{-}Kit^+$ cells as compared to their wild type counterpart treated with PBS; Student's t-Test, n=3. One of two independent experiments is shown.

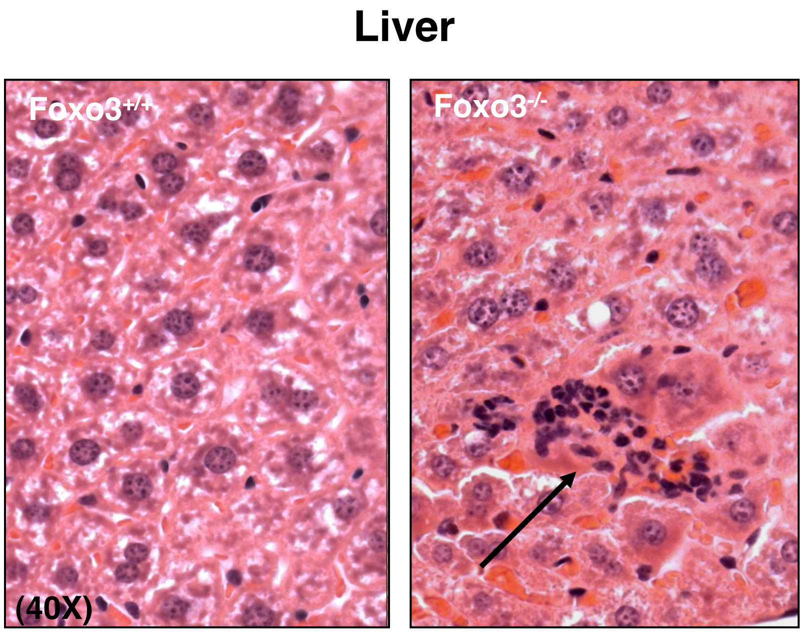
Supplemental Figure 10

Increased Myeloid but not T Cell Lineage in $Foxo3^{-/-}$ Spleens. Frequency of myeloid (Mac-1 and Gr-1 positive) and T (CD4 or CD8 positive) cells in the spleen of wild type or $Foxo3^{-/-}$ mice was analyzed by flow cytometry. Representative results from three independent experiments.

Supplemental Figure 1



Supplemental Figure 2



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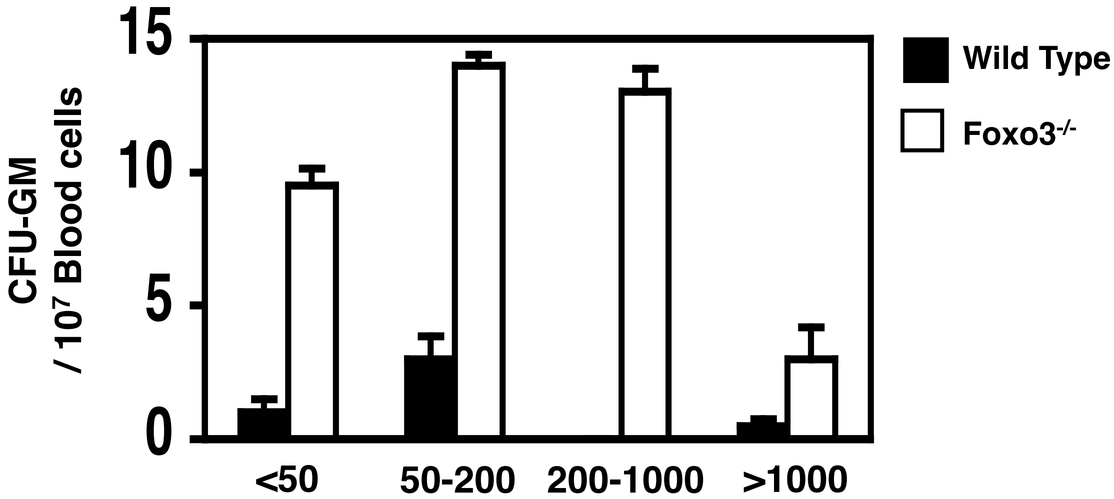

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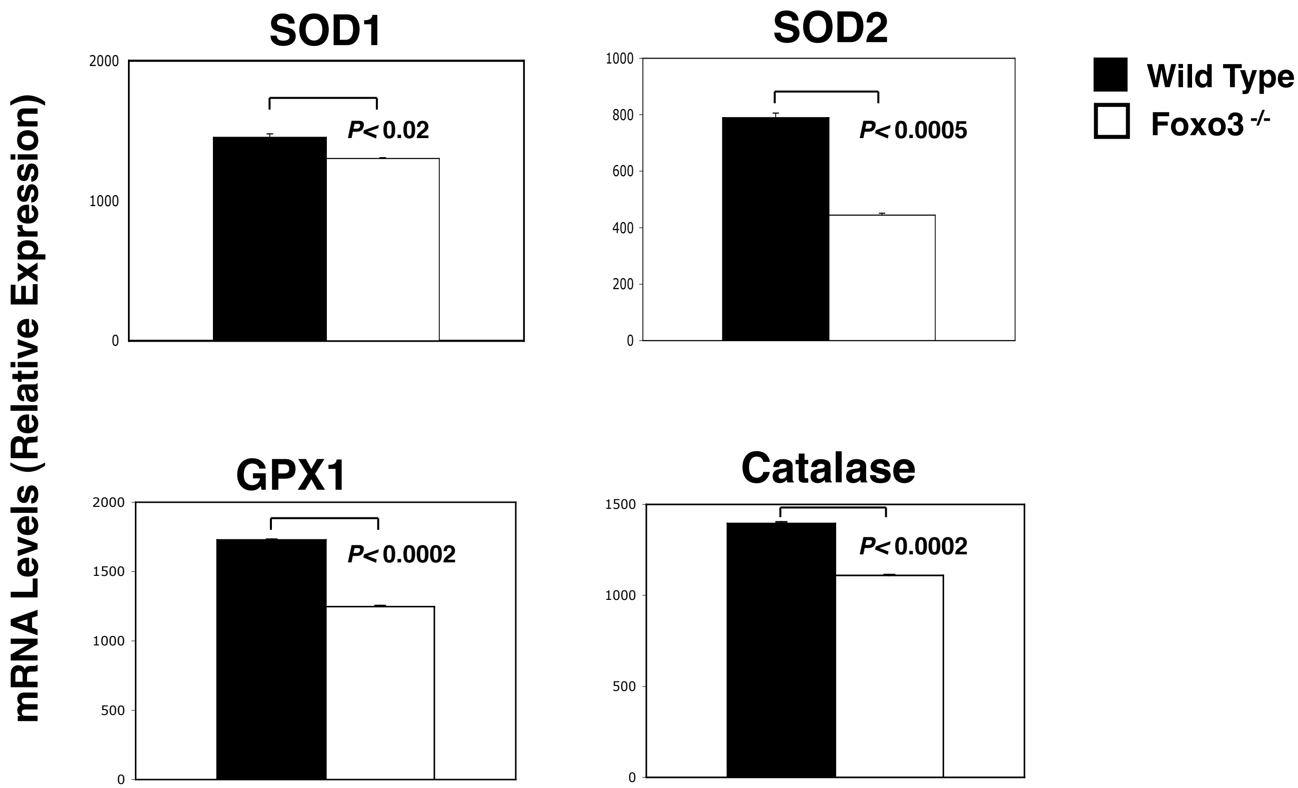

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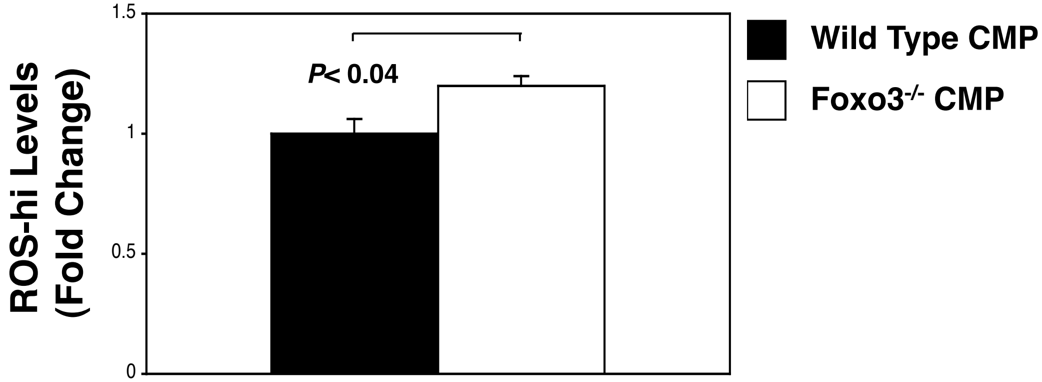

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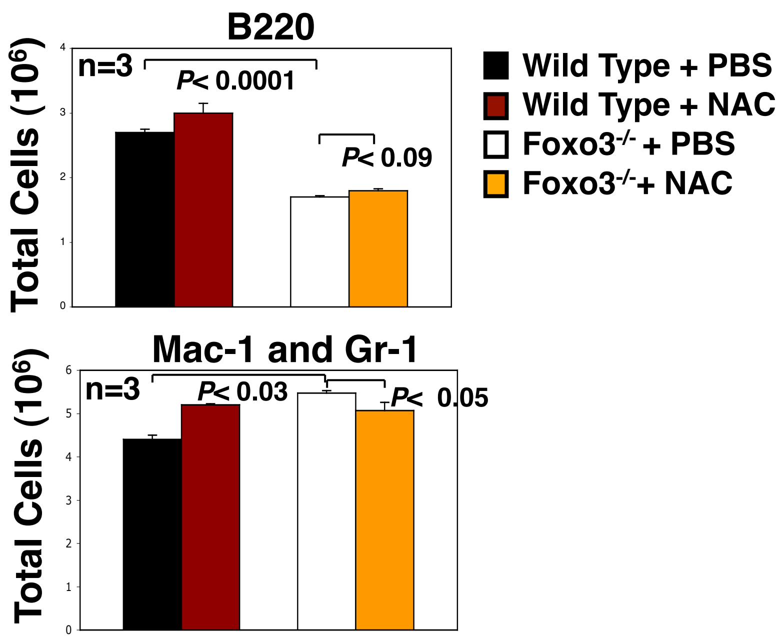
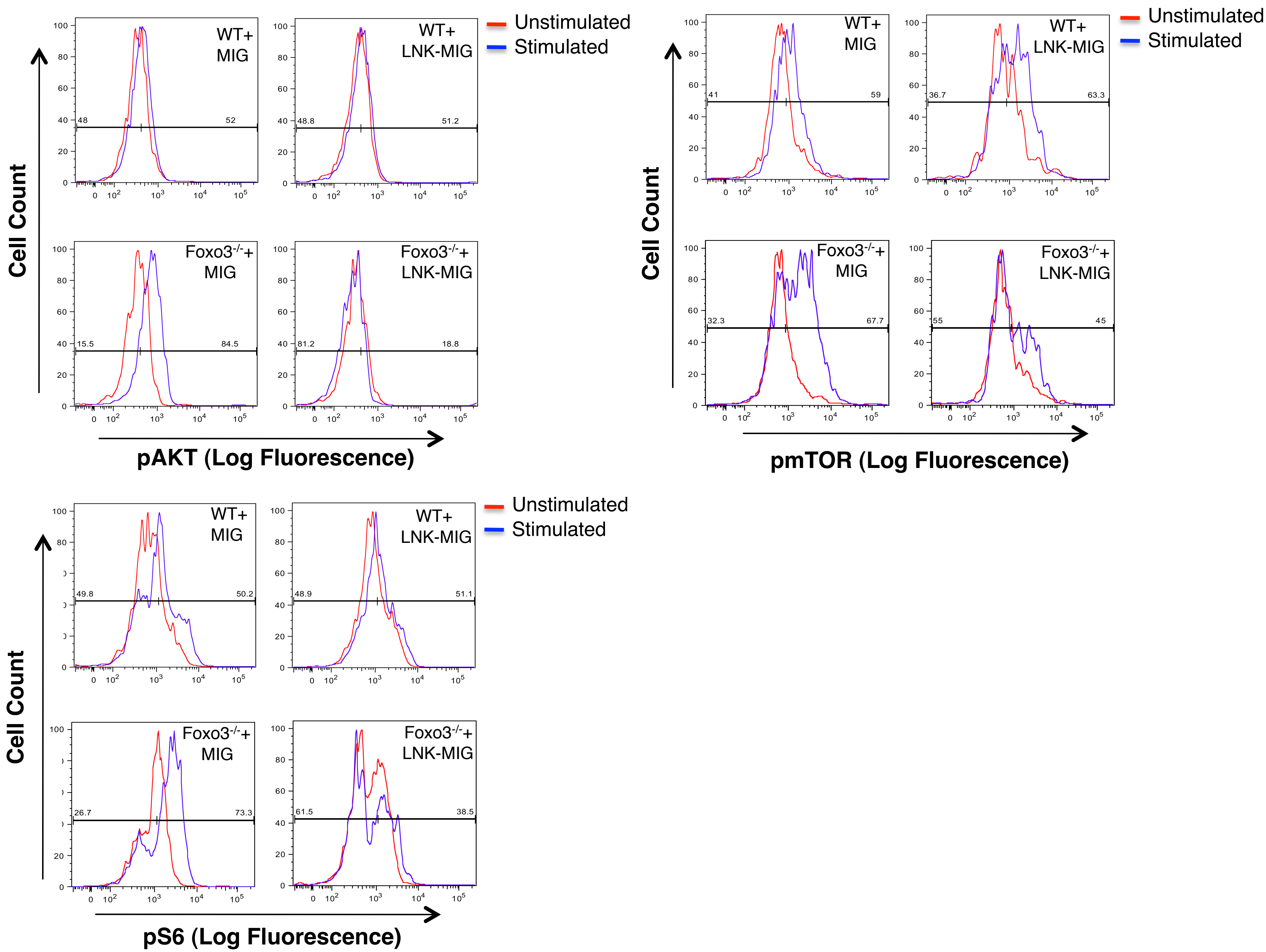
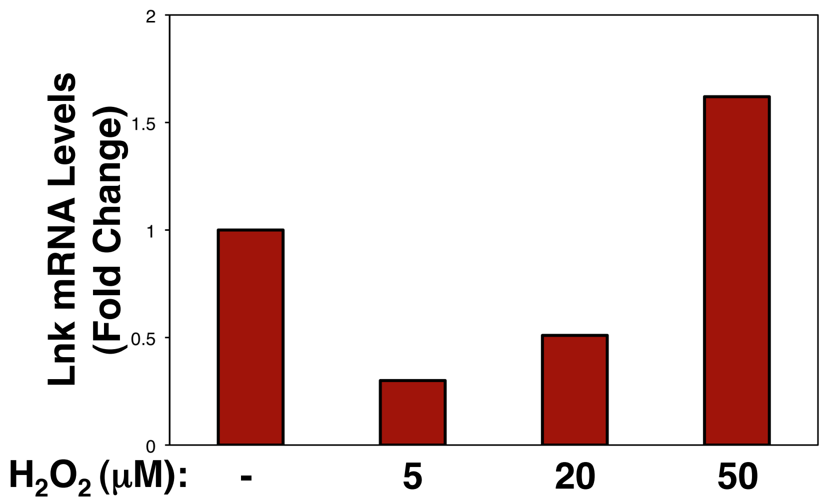


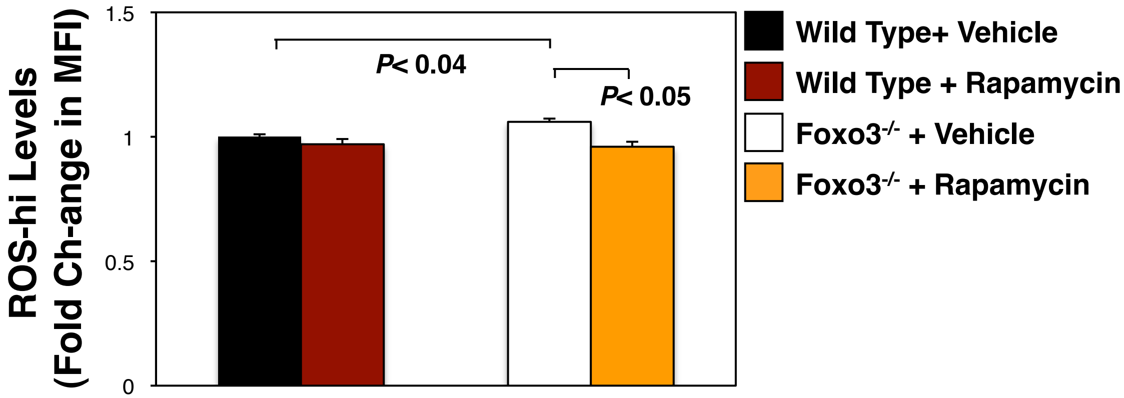
Supplemental Figure 7



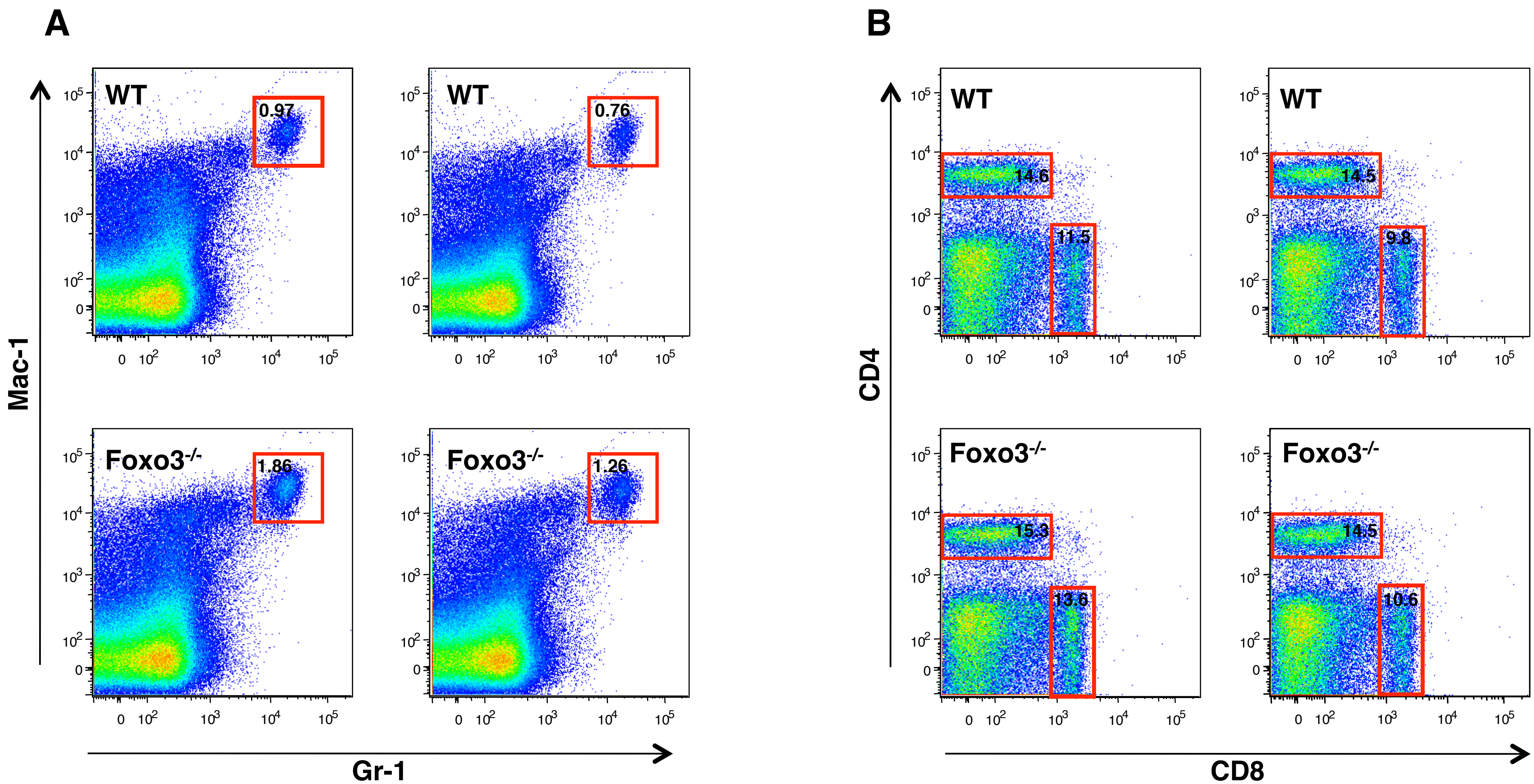
Supplemental Figure 8



Supplemental Figure 9



Supplemental Figure 10



Supplemental Table 1. QRT-PCR Primer Sets

Primer	Forward sequence	Reverse sequence
SH2B1	5'-AGGGACAGTTCATCCCCTCTCTA-3'	5'-CTTCTAACACTAACTGGGCAGCTTT-3'
SH2B2(APS)	5'-GAGTGTGTGCTGACCTTCAACTT-3'	5'-GAAGTGACGGAGCATATCAAACA-3'
SH2B3 (LNK)	5'- AGCCACTTTCTGCAGCTCTTC-3'	5'- GTAGAGGTTGTCAGGCATCTCC-3'
SHIP	5'-GAGGAGGAGGATGCTATTGATG-3'	5'-TCTCTGTTGCAAGAGGAAGGTC-3'
SHP1	5'-GTAAACGCAGCTGACATTGAGA-3'	5'-CCTTCCAGACGTTGGTGTAGAT-3'
SOCS	5'-AGAGAATGAACCGAAGGTGCTA-3'	5'-CTGTCTCGAACTAGGAATGTACCC-3'
PTEN	5'-ATGTGGCGGGACTCTTTGT-3'	5'-AGCGGCTCAACTCTCAAACTT-3'
SOD1	5'-GAGACCTGGGCAATGTGACT-3'	5'-TTGTTTCTCATGGACCACCA-3'
SOD2	5'-TCAATGGTGGGGGACATATT-3'	5'-GAACCTTGGACTCCCACAGA-3'
Catalase	5'-CCTCGTTCAGGATGTGGTTT-3'	5'-TCTGGTGATATCGTGGGTGA-3'
GPX1	5'-GTCCACCGTGTATGCCTTCT-3'	5'-TCTGCAGATCGTTCATCTCG-3'
b-actin	5'-TGTTACCAACTGGGACGACA-3'	5'-GGGGTGTTGAAGGTCTCAAA-3'