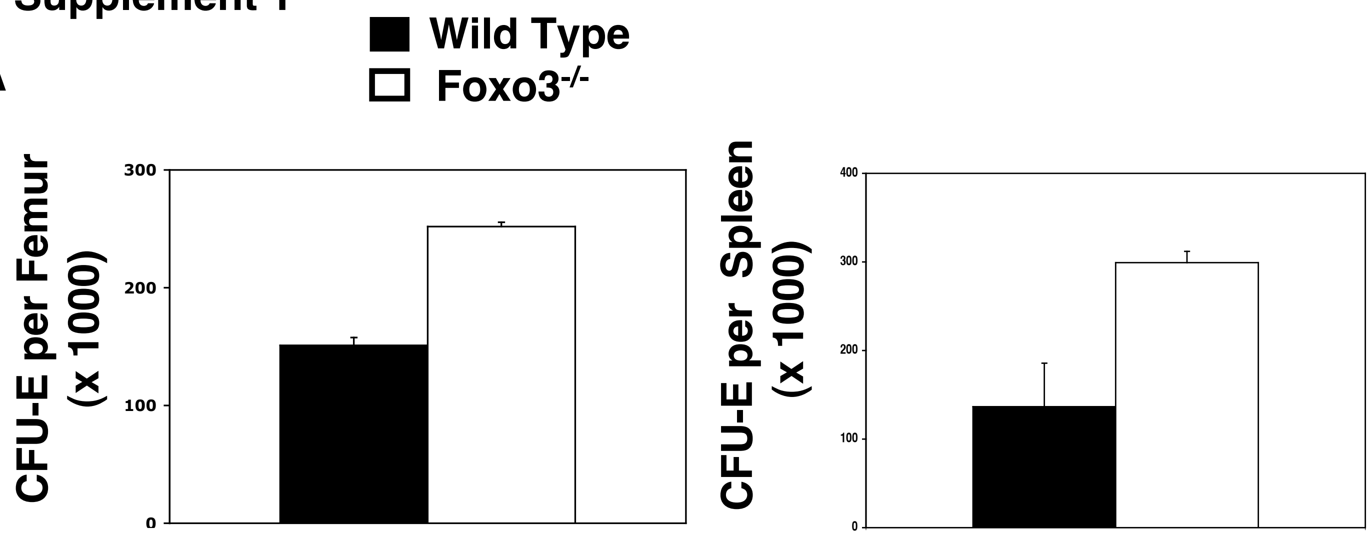
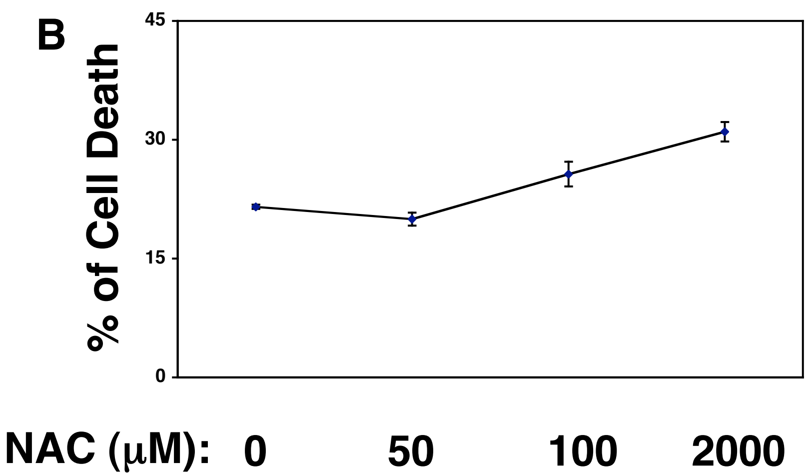
Supplement 1

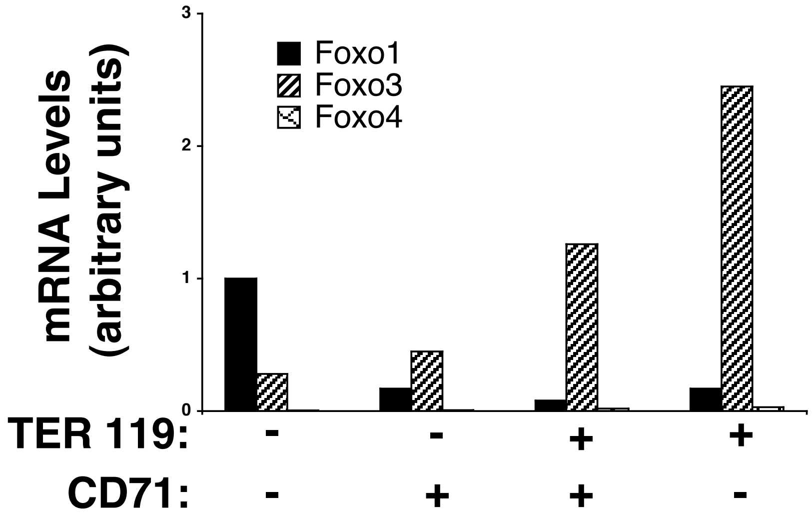
A



B

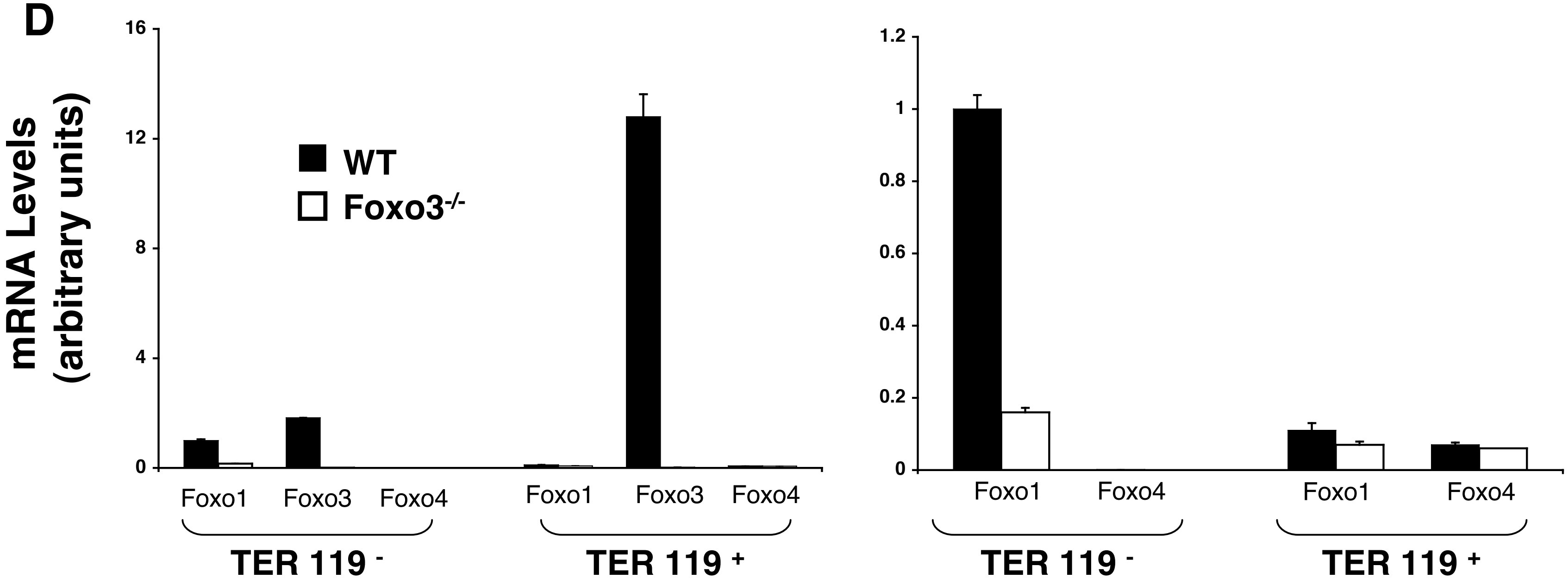


C



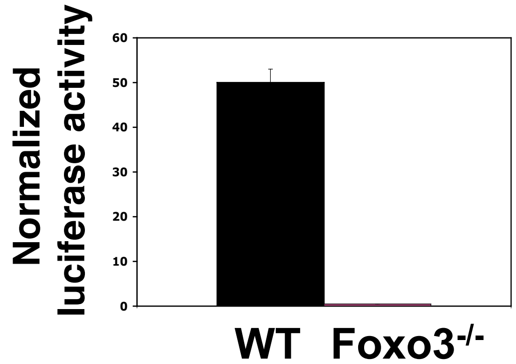
mRNA Levels
(arbitrary units)
3
2
1
0
Foxo1
Foxo3
Foxo4
TER 119:
-
-
+
+
CD71:
-
+
+
-

D

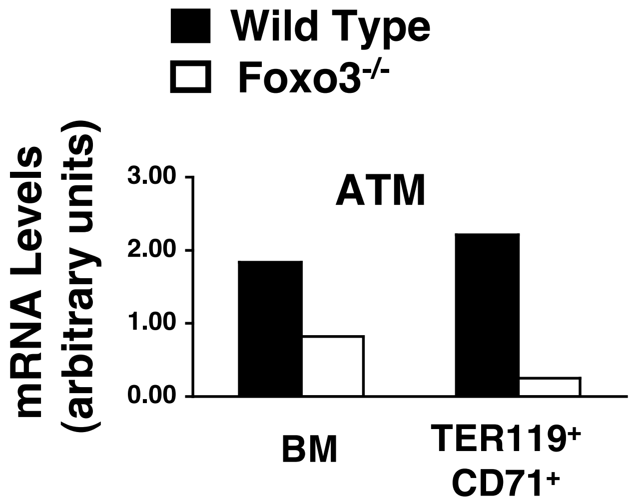


mRNA Levels
(arbitrary units)
16
12
8
4
0
WT
Foxo3-/-
Foxo1
Foxo3
Foxo4
Foxo1
Foxo3
Foxo4
TER 119 -
TER 119 +
1.2
1
0.8
0.6
0.4
0.2
0
Foxo1
Foxo4
Foxo1
Foxo4
TER 119 -
TER 119 +

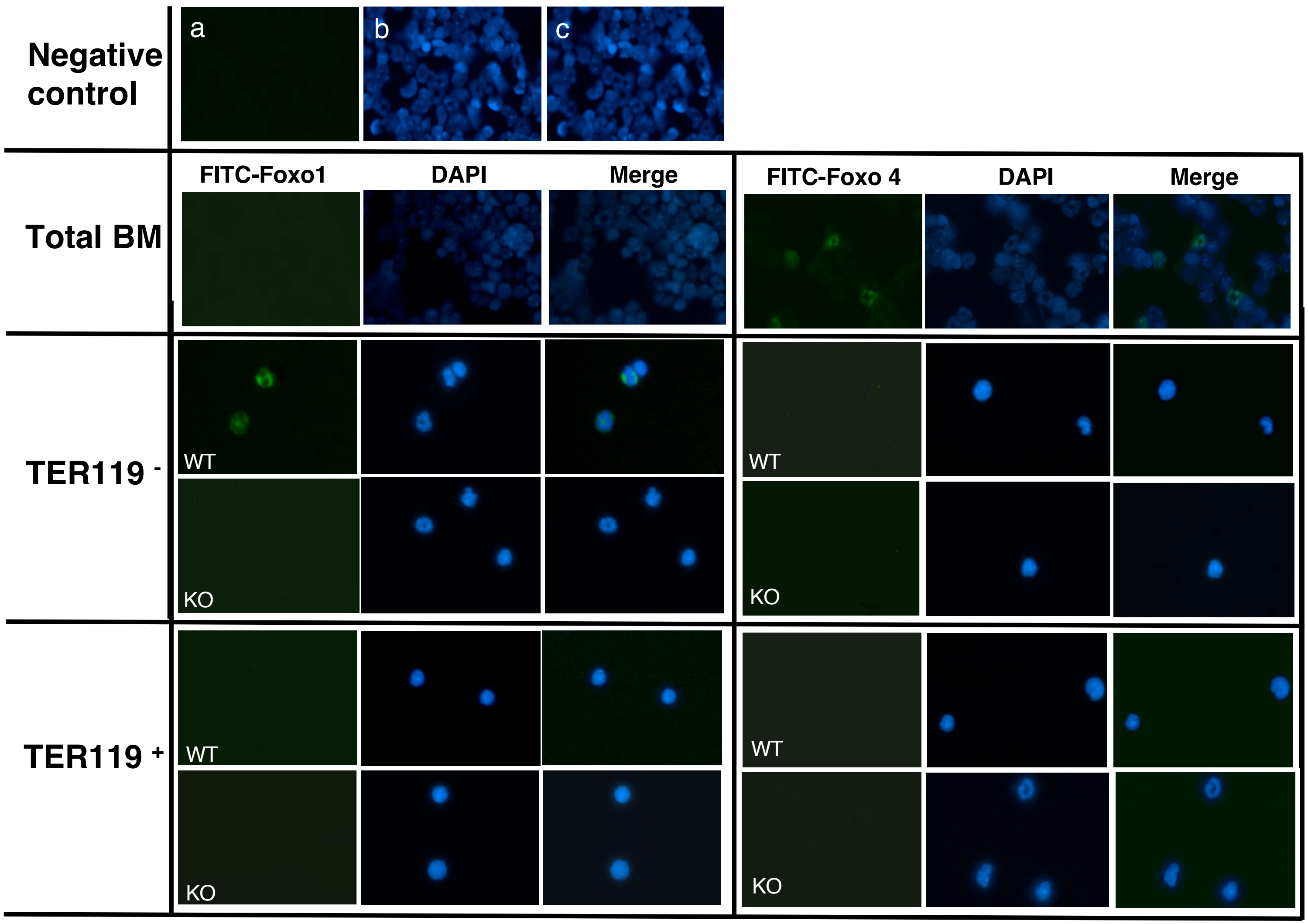
E


Normalized luciferase activity
60
50
40
30
20
10
0
WT
Foxo3-/-

F


Wild Type
Foxo3-/-
mRNA Levels (arbitrary units)
3.00
2.00
1.00
0.00
ATM
BM
TER119+ CD71+

G



Negative control
a
b
c
Total BM
FITC-Foxo1
DAPI
Merge
FITC-Foxo 4
DAPI
Merge
TER119 -
WT
KO
WT
KO
TER119 +
WT
KO
WT
KO

Supplement 1

A. Erythroid hyperplasia in Foxo3-deficient spleen. Total number of mature erythroid colony CFU-E in the bone marrow and spleen of wild type and Foxo3-deficient mice. Erythroid colony assays were performed as previously described (39) in the presence of Epo (0.3 Units/ml) and rat stem cell factor (SCF, PeproTech, NJ) 100 ng/ml.

B. Relative toxicity of NAC treatment on hematopoietic cells. Bone marrow cells were incubated with or without NAC at the indicated doses under optimum growth factor conditions in vitro for 48 hours and cell death was measured by trypan blue staining. Results are shown as mean ± SEM of 8 distinct bone marrows (from 8 mice).

C. QRT-PCR analysis of Foxo expression in fetal liver. Total RNA extracted from FACS sorted subpopulations of E14.5 fetal liver cells was subjected to QRT-PCR analysis of Foxo expression. Results shown relative to Foxo1 expression in $CD71^{-}$ TER 119^{-} cells (equal to 1).

D. QRT-PCR analysis of Foxo expression in wild type and Foxo3-deficient bone marrow. Total RNA extracted from FACS sorted TER 119^{-} and TER 119^{+} subpopulations of wild type and Foxo3-deficient bone marrow was subjected to QRT-PCR analysis of Foxo expression. Results shown relative to Foxo1 expression in wild type TER 119$^-$ cells (equal to 1). Note the relative low expression of Foxo1 and Foxo4 as compared to Foxo3.

E. Luciferase activity of Foxo3 reporter in Foxo3-deficient mouse embryonic fibroblasts (MEF). Wild type and Foxo3-deficient MEFs were transfected by calcium phosphate with a luciferase reporter construct containing five tandem repeat of consensus Foxo binding sites (pTA-Foxo5BS-Luc) and luciferase activity was measured at 48 hours.

F. ATM is downregulated in the erythroid precursor subpopulation. QRT-PCR expression of ATM in total and erythroid precursors in the wild type and Foxo3-deficient bone marrow.

G. Immunostaining of Foxo in TER 119$^+$ and TER 119$^-$ subpopulations of bone marrow. Immunofluorescent staining of Foxo1 (using anti-Foxo1, Santa Cruz) and Foxo4 (using anti-Foxo4, Santa Cruz) in FACS sorted TER 119$^-$ and TER 119$^+$ subpopulations of wild type and Foxo3-deficient bone marrow. Total wild type bone marrow cells were used as positive controls. TER 119$^-$ wild type bone marrow stained with anti-goat IgG (a), DAPI (b), merged (c). Note absence of Foxo1 staining in total bone marrow consistent with the Western blot analysis (Figure 4B). Note also lack of Foxo1 and Foxo4 expression in wild type or Foxo3-deficient TER 119$^+$ bone marrow cells.

Supplement Table 2

Primer	Forward sequence	Reverse sequence
Foxo1	5'-AGCTGGGTGTCAGGCTAAGA-3'	5'-TGGACTGCTCCTCAGTTCCT-3'
Foxo3	5'-TCGAAGTGGAGCTGGACCC-3'	5'-TGCCGGCGTCTGAGGTACTA-3'
Foxo4	5'-CAGTGACCTCATGGATGGTG-3'	5'-TGCTGTGACTCAGGGATCTG-3'
α-globin	5'-GCTGAAGCCCTGGAAAGGAT-3'	5'-TGGCTTACATCAAAGTGAGGAAAG-3'
β-globin	5'-TGCCTCTGCTATCATGGGTAATG-3'	5'-GGCCATCGTTAAAGGCAGTTAT-3'
GATA-1	5'-TGCCCCAGTTTGTGGATTCT-3'	5'-CCCTCTGGCCCAGAGGAA-3'
GATA-2	5'-ATACCCACCTATCCCTCCTATGTG-3'	5'-GCCTCCTGGATGGAAGAGACT-3'
EKLF	5'-TGCCCACGTGCTTTTTCAC-3'	5'-TCCTTGTGCAGGATCACTCAGA-3'
HO-1	5'-AAGAGGCTAAGACCGCCTTC-3'	5'-TCCTCTGTCAGCATCACCTG-3'
HO-2	5'-TGGCACCAGAAAAGGAAAAC-3'	5'-CTTCCTTGGTCCCTTCCTTC-3'
ATM	5'-CCAGGGGAAGATGATGAAGA-3'	5'-TCGGCAGCTAAAGGACTCAT-3'
SESN2	5'- TAGCCTGCAGCCTCACCTAT-3'	5'- GATTTTGAGGTTCCGTTCCA-3'
GADD45a	5'-TGAGCTGCTGCTACTGGAGA-3'	5'-TCCCGGCAAAAACAAATAAG-3'
Cyclin G2	5'-CAGGACAGGTGTTTCGGTTT-3'	5'-AAAGGGCTGATCTTGATGGA-3'
p53	5'-GATGACTGCCATGGAGGAGT-3'	5'-GTCCATGCAGTGAGGTGATG-3'
p21	5'-GTACTTCCTCTGCCCTGCTG-3'	5'-TCTGCGCTTGGAGTGATAGA-3'
p27	5'-AGGAGAGCCAGGATGTCAGC-3'	5'-CAGAGTTTGCCTGAGACCCAA-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'
β-actin	5'-TGTTACCAACTGGGACGACA-3'	5'-GGGGTGTTGAAGGTCTCAAA-3'