Expanded View Figures

Figure EV1. ROS levels and mitochondrial membrane potential in HSPC.
Endogenous ROS levels were measured in WT and Foxo3−/− LSK cells by flow cytometry.
A, B Representative FACS histogram (left panel) and geometric mean fluorescence intensity normalized with WT (right panel) of ROS levels by chloromethyl dichlorodihydrofluorescein diacetate, CM-H2DCFDA (A), and mitoSOX Red (B) fluorescence are shown. One representative of three independent experiments (n = 3 mice per genotype) is shown.
C, D Representative FACS histograms of DiIC1(5) (C) and contour plots of JC-1 red and green fluorescence (D) in WT LSK treated (CCCP) or not (WT) with a ΔΨm inhibitor CCCP (carbonyl cyanide 3-chlorophenylhydrazone) 50 mM in vitro for 20 min.
E Histogram of TMRE fluorescence displaying shifts in fluorescence intensity after treatment with either CCCP or oligomycin in BM cells.
Figure EV2. Mitochondrial morphology in Foxo3−/− LSK cells.

A. FACS-sorted LSK cells (10,000 cells) cytospun onto slides and stained with TOM20 (mitochondria-specific antibody) and a secondary conjugated to Alexa Fluor 488 and mounted with DAPI (60×). Images analyzed using ImageJ and the Mito-Morphology macro [77]. Representative images are displayed with nucleus in blue and mitochondria in red. Outlines of mitochondrial networks based on TOM20 fluorescence intensity set at a threshold ≥ 170 a.u. are shown.

B. Quantification of perimeter and area of distinct mitochondrial networks using the Mito-Morphology macro. The average mitochondrial perimeter (left) or area (right) per cell analyzed and error bars represent SEM. *P < 0.05, Student’s t-test.

C. The circularity (left) of mitochondria was quantified, with higher values representing more circular mitochondria. Quantification of mitochondrial network interconnectivity (right) based on a ratio of the area to the perimeter, normalized to the circularity. Bars represent mean ± SEM. *P < 0.05, Student’s t-test.
Figure EV3. Oxyblot of NAC-treated BM cells. (Top) WT and Foxo3−/− mice treated with either PBS or NAC were sacrificed, and protein isolated from total BM cells was subjected to protein oxidation analysis. Blots comparing extent of protein oxidation in PBS-treated animals (left blot) and NAC-treated animals (right blot). Each number represents a single mouse with DNPH (+)-treated and DNPH (−)-negative controls in adjacent lanes. (Bottom) Quantification of oxidized proteins detected by oxyblot comparing WT and Foxo3−/− mice treated with vehicle (PBS) or NAC. Values were normalized to DNPH (−) controls. Bars represent mean ± SEM. n.s., not significant; *P < 0.05, Student's t-test.
Figure EV4. Effects of NAC on HSPC ROS and mitochondrial membrane potential.

A. Quantification of ROS levels by DCF probe in LT-HSC after 4-week treatment with NAC or vehicle control prior to transplantation. Bars represent DCF fluorescence (mean ± SEM, n = 3). *P < 0.05, Student’s t-test.

B. Quantification of ROS levels in donor-derived peripheral blood cells at 16 weeks post-transplantation comparing all combinations of post- and pre-transplantation treatment with NAC. 16-week PB donor cells (ROS), post-transplant vehicle: WT-C = 4, KO-C = 4, WT-NAC = 5, KO-NAC=3; post-transplant NAC: WT-C = 4, KO-C = 3, WT-NAC = 3, KO-NAC = 1. Bars represent mean ± SEM.

C. Mitochondrial membrane potential quantification with TMRE in LSK cells. TMRE fluorescence (mean ± SEM), n = 3. n.s., not significant; *P < 0.05, Student’s t-test.

D. Quantification of ROS levels by DCF probe in LSK cells after treatment with NAC or vehicle control. Bars represent DCF fluorescence (mean ± SEM). *P < 0.05, Student’s t-test.