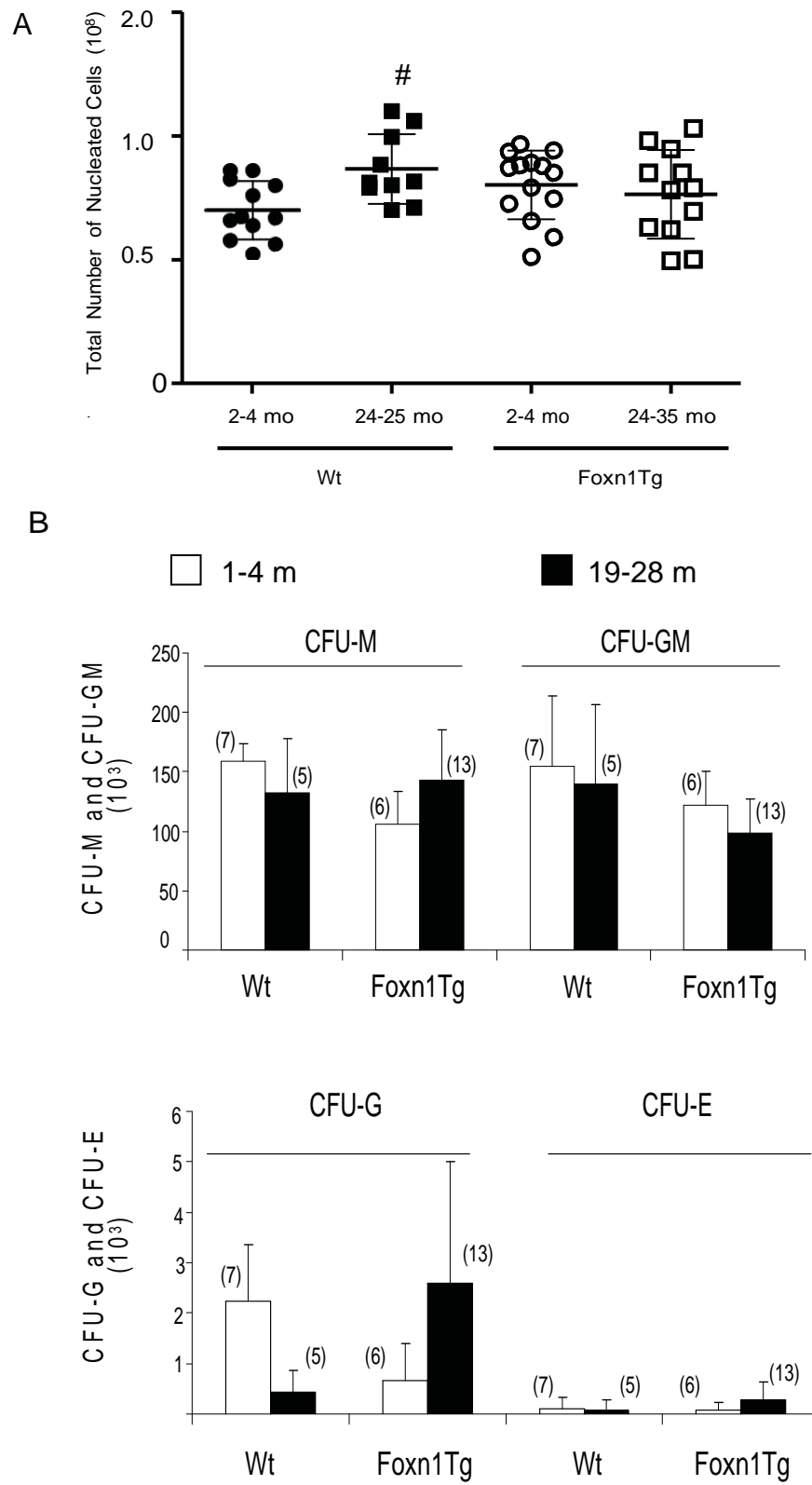
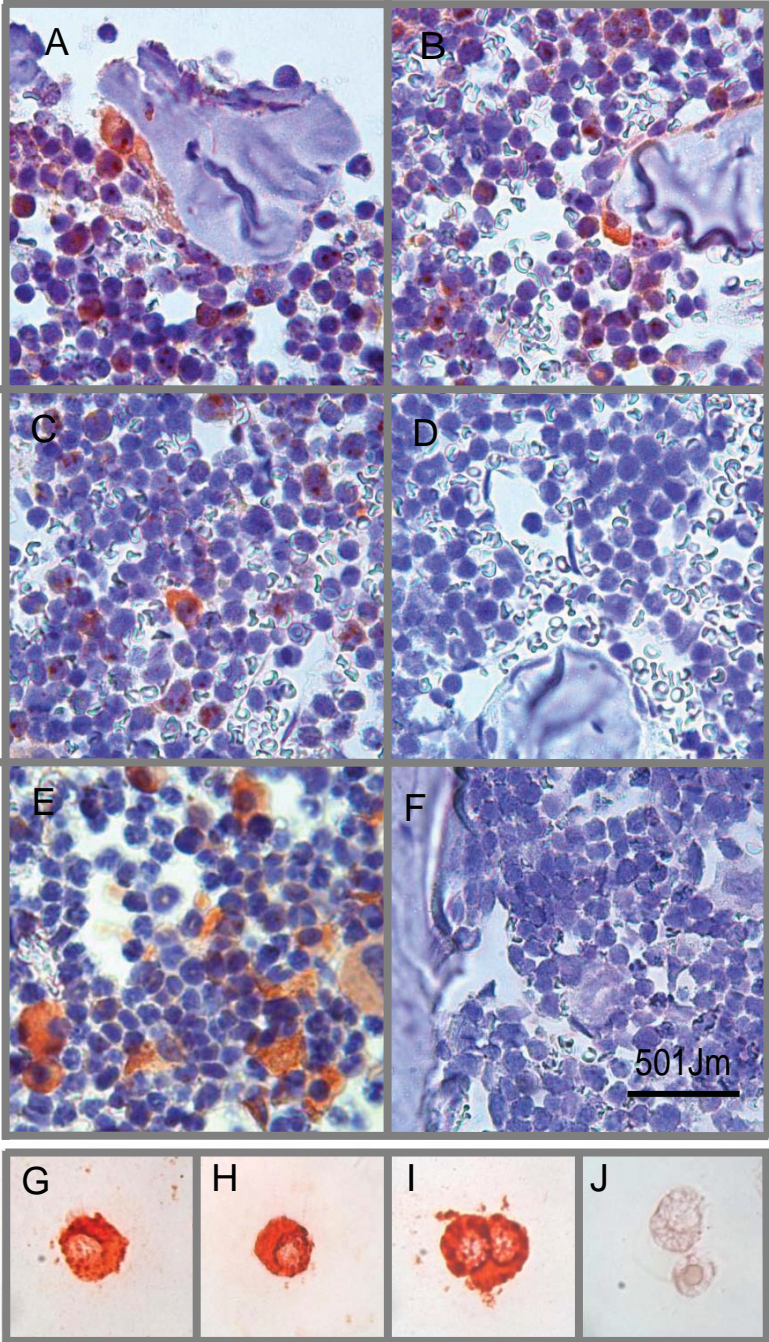
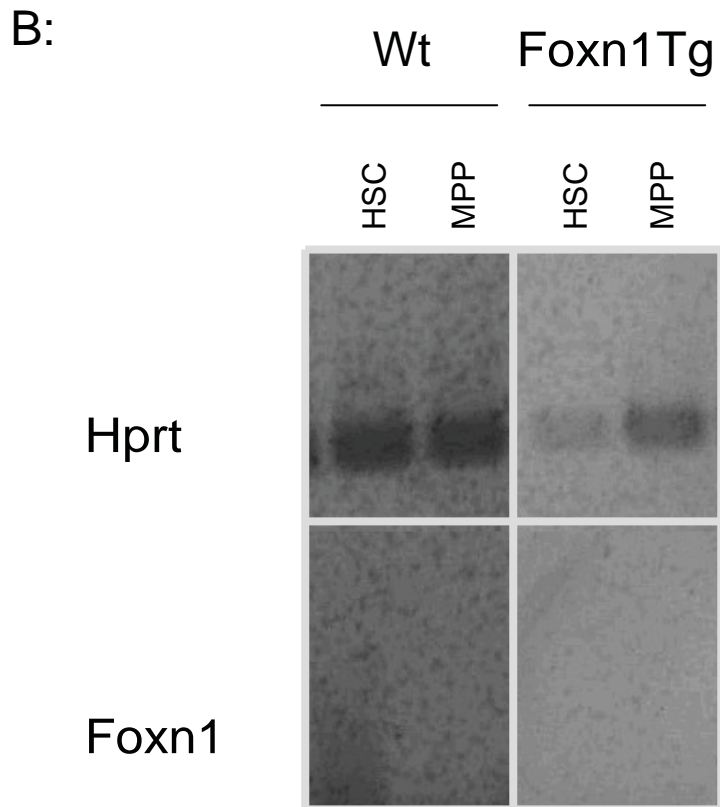
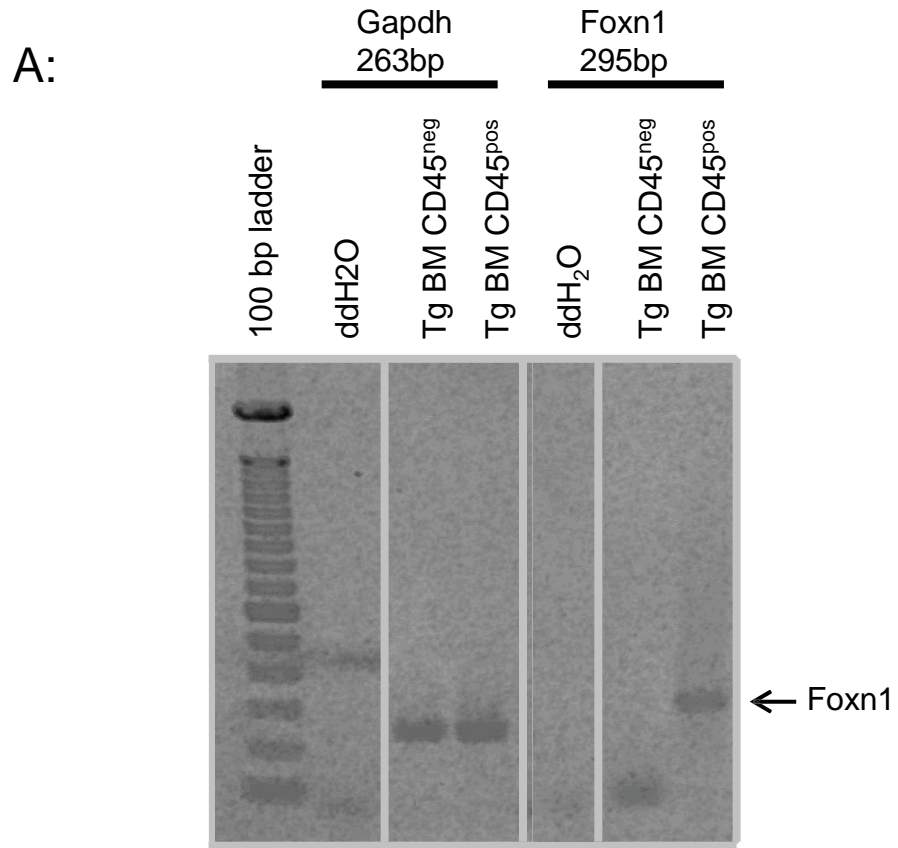


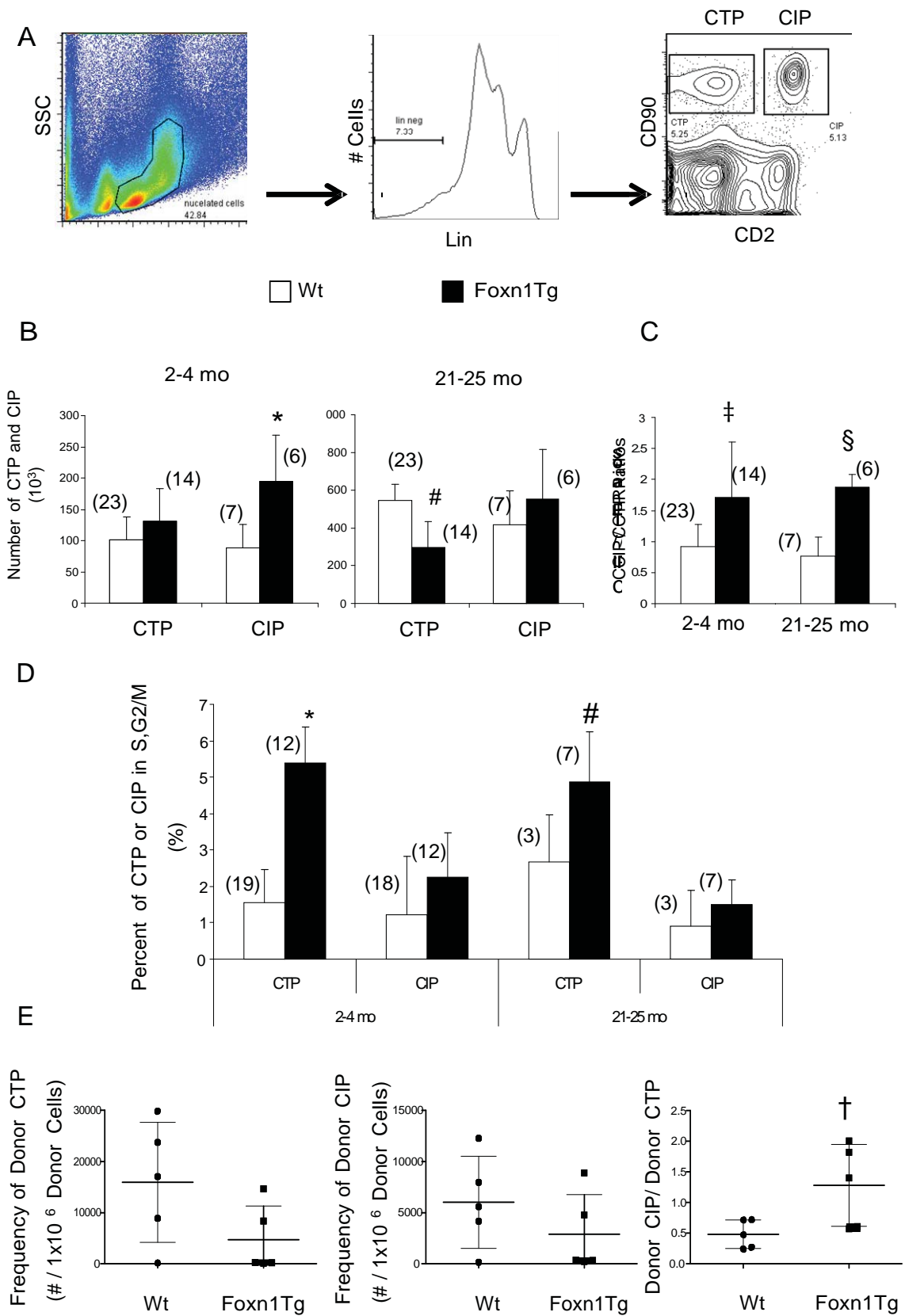
Zook et. al  
 Supplemental  
 Figure 1







Supplemental Figure 4



## Figure Legends

Supplemental Figure 1. A Total number of nucleated cells in the BM of young and aged Wt and Foxn1Tg. Two tibias and femurs were flushed and the total number of nucleated cells in Wt, (closed circles 1-4 mo and closed squares 24-25 mo), and Foxn1Tg (open circles 1-4 mo and open squares 24-35 mo) were counted using a hemocytometer. P values were from t-test; #p=0.01 Wt 1-2 mo vs. Wt 24-25 mo. Each symbol represents result from one individual animal from 3-4 experiments. Error bars are SD. B. In vitro generation of CFU-M, CFU-GM, CFU-G, and CFU-E colonies in young and aged Wt and Foxn1Tg BM. Total number of CFU-M, CFU-GM, CFU-G and CFU-E in Wt and Foxn1Tg 1-4 (white bars) and 19-28 (black bars) months of age. Numbers in parentheses denotes the number of mice. Error bars are SD.

Supplemental Figure 2: K14 expression in the BM of WT and Foxn1Tg mice. Keratin 14 expressing cells in the BM as determined by immunohistochemistry assay. Sternums from Wt and Foxn1Tg mice were fixed, embedded in paraffin blocks, and sectioned at 5  $\mu$ m. Antigen retrieval was performed on rehydrated tissue sections prior to staining with either rabbit anti-mouse K14 (or rabbit IgG at 2!-g/ml. Primary antibodies were incubated overnight at 4°C. Dako Universal LSAB biotinylated antibody cocktail or Donkey anti-rabbit biotin (6 !-g/ml) followed by streptavidin-HRP was used for detection of primary antibody. Sections were developed with AEC for 1.5 minutes and counterstained with hematoxylin. Pictures were taken using a Leitz Diaplan microscope

with Retiga 2000R camera. A-D: Wt with anti-K14 antibody (A-C) and control IgG (D). E, F: Foxn1Tg with anti-K14 antibody (E) and IgG (F). G-J: Sorted Lin<sup>neg</sup> EpCAM<sup>pos</sup> CD138<sup>neg</sup> cells from Foxn1Tg BM stained with anti-K14 (G-I) or rabbit IgG control (J).

Supplemental Figure 3: Foxn1 is expressed in CD45<sup>pos</sup> hematopoietic cells but is not expressed in HSC and MPP. A) Tibias and femurs from a Foxn1Tg mouse were flushed and BM cells were sorted for CD45<sup>pos</sup> and CD45<sup>neg</sup> subsets. RT-PCR was used to measure if Foxn1 expressed in CD45<sup>neg</sup> and CD45<sup>pos</sup> cells. Vertical lines represent repositioning of gel. B) HSC and MPP were electronically sorted from the BM of Wt and Foxn1Tg mice and RT-PCR was used to determine if HSC and MPP express Foxn1.

Supplemental Figure 4: Assessment of CTP and CIP: A-D, Changes in number and cell cycle profile with age; E, generation of CTP and CIP populations after adoptive transfer into Wt and Foxn1Tg hosts. Flow cytometry was used to determine the total number of CTP and CIP in the BM of young (2-4 mo) and aged (21-25 mo) Wt and Foxn1Tg mice. A) Flow cytometry gating of CTP and CIP. BM CTP were identified as Lin<sup>neg</sup> CD90<sup>pos</sup> CD2<sup>neg</sup> and CIP as identified as Lin<sup>neg</sup> CD90<sup>pos</sup> CD2<sup>pos</sup>. CTP and CIP frequencies were used to calculate the total number of each population based on the number of nucleated cells isolated from 2 tibias and 2 femurs. B) Total CTP and CIP in young (2-4 mo) and aged (21-25 mo) Foxn1Tg and Wt mice; \*p<0.001 CIP in Wt vs. Foxn1Tg. The total numbers of CTP and CIP increased with age in both Wt and

Foxn1Tg ( $p < 0.001$  Wt CTP 2-4 mo vs. Wt CTP 21-25 mo;  $p = 0.006$  Foxn1Tg CTP 2-4 mo vs. Foxn1Tg CTP 21-25 mo;  $p < 0.001$  Wt CIP 2-4 mo vs. Wt CIP 21-25 mo;  $p = 0.002$  Foxn1Tg CIP 2-4 mo vs. Foxn1Tg CIP 21-25 mo). Aged Foxn1Tg 21-25 mo had fewer CTP than Wt 21-25 mo,  $^{\#}p = 0.002$  vs. Foxn1Tg. C) Ratio of CIP over CTP in Wt and Foxn1Tg;  $^{\dagger}p = 0.047$  and  $^{\S}p < 0.001$  were comparisons of Wt vs. Foxn1Tg. D) Electronically sorted CTP and CIP from young (2-4 mo) and aged (21-25 mo) Wt and Foxn1Tg were analyzed for DNA content using propidium iodine and flow cytometry. Data represent the percentages of cells in S,G2/M phase.  $^*p = 0.002$  CTP from Foxn1Tg 2-4 mo vs. Wt 2-4 mo;  $^{\#}p = 0.054$  CTP from Foxn1Tg 21-25 mo vs. Wt 21-25 mo. Numbers in parentheses denote number of mice in each age group. Error bars are SD; p values were from t-test. E) Data were obtained from adoptive transfer experiments described in Figure 5 and analyzed for the frequencies of donor CTP, CIP and ratios of CIP/CTP. Each symbol represents result from one mouse. Error bars are SD.