SUPPLEMENTARY DATA

Osteoblastic Wls Ablation Protects Mice from Total Body Irradiation–Induced Impairments in Hematopoiesis and Bone Marrow Microenvironment

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Supplementary Figure 1. Osteoblastic Wls deletion does not alter BM number of HSCs and expression levels of oxidative stress- or senescence-specific markers in these cells at 4 weeks of age. (A) BM frequency of HSCs and numbers of HSCs positive to (B) MitoSox, (C) C12:FDG, or (D) p16INK4a in Wls<sup>fl/fl</sup> and Col2.3-Cre;Wls<sup>fl/fl</sup> mice at 4 weeks of age were analyzed by flow cytometry (n = 10). The significant differences were determined by unpaired Student t-test. ns, not significant.
Supplementary Figure 2. Osteoblastic Wls ablation does not change hematopoietic development and colony forming potentials of HPCs at young stage. Numbers of (A) GMP, (B) CMP, (C) MEP, and (D) CLP cells in Wls^+/+ and Col2.3-Cre;Wls^+/+ mice at 4 weeks of age were determined by flow cytometry (n = 7). At the same age, proportions of peripheral cells positive to (E) Gr-1, (F) CD11b, (G) CD3, or (H) B220 were determined (n = 7). In addition, BM HPCs isolated from Wls^-/- and Col2.3-Cre;Wls^-/- mice at 4 weeks of age were cultured in methylcellulose-based medium. After 12 days of incubation, numbers of (I) CFU-GM, (J) BFU-E, and (K) CFU-GEMM were counted (n = 5). The significant differences in panels A–H were determined by unpaired Student t-test, whereas the values in panels I–K were by non-parametric Wilcoxon t-test. ns, not significant.
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Supplementary Figure 3. A schematic illustration exhibiting serial transplantation of BM HSCs into lethally irradiated recipients for survival assay.

Supplementary Figure 4. TBI does not induce any acute changes in numbers of BM HSCs and MSCs regardless of the presence and absence of osteoblastic Wls. Wls$^{fl/fl}$ and Col2.3-Cre;Wls$^{fl/fl}$ mice at 4 weeks of age were exposed to sub-lethal TBI. The frequencies of (A) BM HSCs and (B) MSCs in these mice were determined by flow cytometry at the indicated times following TBI (n = 5). The significant differences were determined by non-parametric Wilcoxon t-test. ns, not significant.
Supplementary Figure 5. TBI does not affect the expression and secretion of DKK1 in the BM of Wls\textsuperscript{fl/fl} and Col2.3-Cre;Wls\textsuperscript{fl/fl} mice. (A) Wls\textsuperscript{fl/fl} and Col2.3-Cre;Wls\textsuperscript{fl/fl} mice at 4 weeks of age were exposed to 5 Gy TBI, and expression pattern of DKK1 in the BM of these mice was evaluated by IHC assay at the indicated times after TBI. Scale bars = 100 μm. (B) The area (%) positively stained with DKK1 in IHC assay was calculated (n = 5). (C) Protein level of DKK1 in whole BM lysate of the control and mutant mice was determined by ELISA at the indicated times after TBI (n = 5). The significant differences were determined by non-parametric Wilcoxon t-test. ns, not significant.
Supplementary Figure 6. The BM level of FGF21 was not changed by osteoblastic Wls ablation or in combination with sublethal TBI. (A) Wls\(^{−/−}\) and Col2.3-Cre;Wls\(^{−/−}\) mice at 4 weeks of age were exposed to 5 Gy TBI, and expression level of FGF21 in the BM of these mice was evaluated by IHC assay at 5 days post-TBI. Scale bars = 200 μm. (B) The quantified DAB intensity specific to FGF21 in the images was converted to optical density (OD) using the following formula: OD = log (max intensity/mean intensity), where max intensity = 255 for 8-bit images (n = 5). The significant differences were determined by non-parametric Wilcoxon t-test. ns, not significant.