An abnormal bone marrow microenvironment contributes to hematopoietic dysfunction in Fanconi anemia

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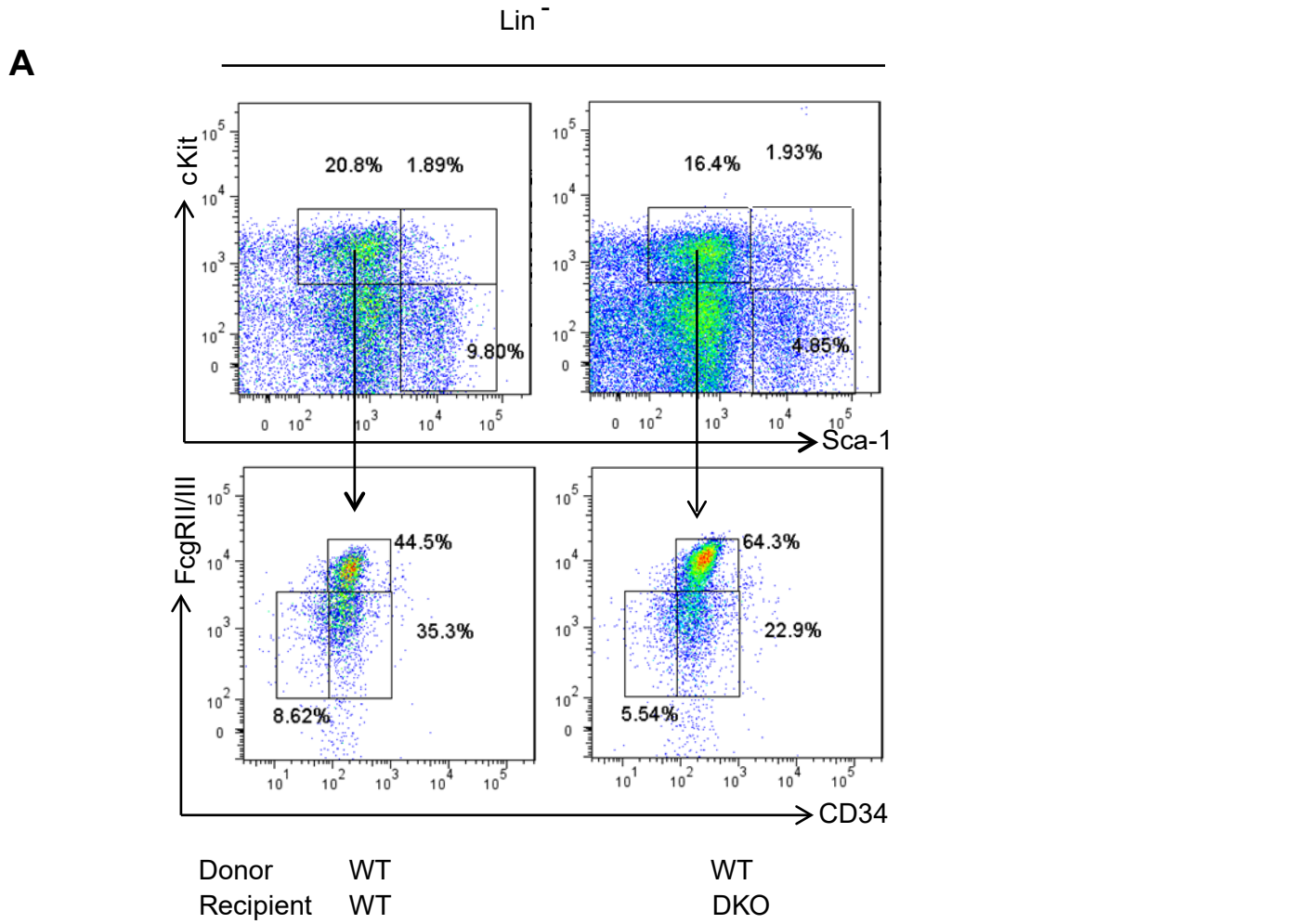
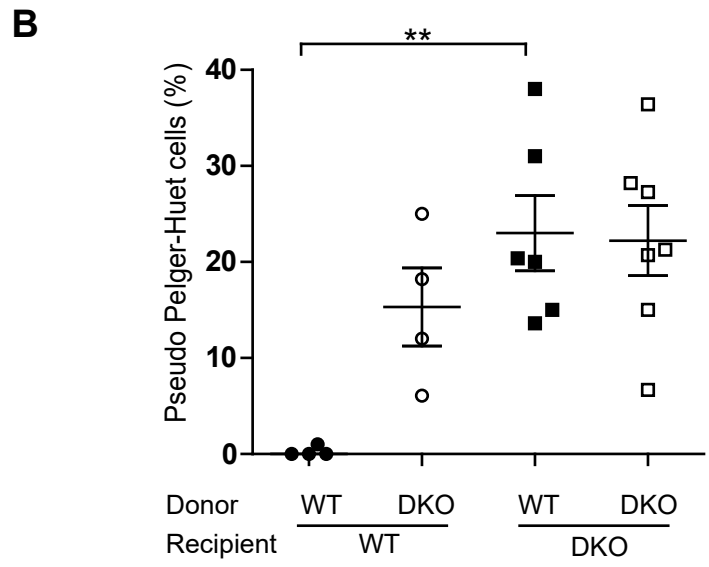
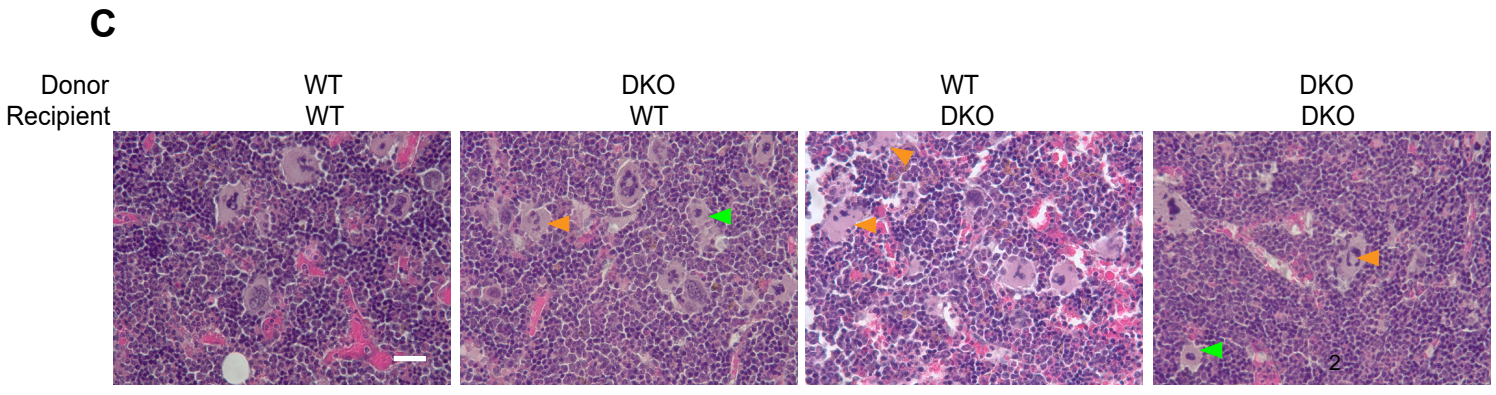
Supplementary Figure 1.

(A) DKO and WT recipient mice were transplanted with bone marrow cells from WT donor mice. Six months after transplantation, the mice were sacrificed, and the BMMNCs were processed for analysis. Multi-color flow cytometry showed the altered distribution of myeloid progenitors in DKO recipient mice transplanted with WT hematopoietic cells, as compared to WT recipients. $Lin^-Sca\text{-}1^-c\text{-}Kit^+$ progenitor cells were split into common myeloid progenitors ($lin^-Sca\text{-}1^-c\text{-}Kit^+FcgRII/III^-CD34^+$), myeloid-erythroid progenitors ($lin^-Sca\text{-}1^-c\text{-}Kit^+FcgRII/III^-CD34^-$) and granulocyte -macrophage progenitors ($lin^-Sca\text{-}1^-c\text{-}Kit^+FcgRII/III^+CD34^+$). **(B)** Increased percentage of pseudo Pegler-Huet cells in DKO recipient mice transplanted with WT bone marrow cells compared with WT recipient. Data are presented as mean ± SEM, $^{**}P < 0.01$, 2-tailed Student's *t*-test. **(C)** Representative photomicrographs demonstrated features of megakaryocytes 15 months post-transplantation at high (40×) magnification. The Orange arrow indicated multinuclear megakaryocytes and a green arrow indicated hyposegmented megakaryocyte. Scale bar: 10 μm.

Supplementary Figure 1



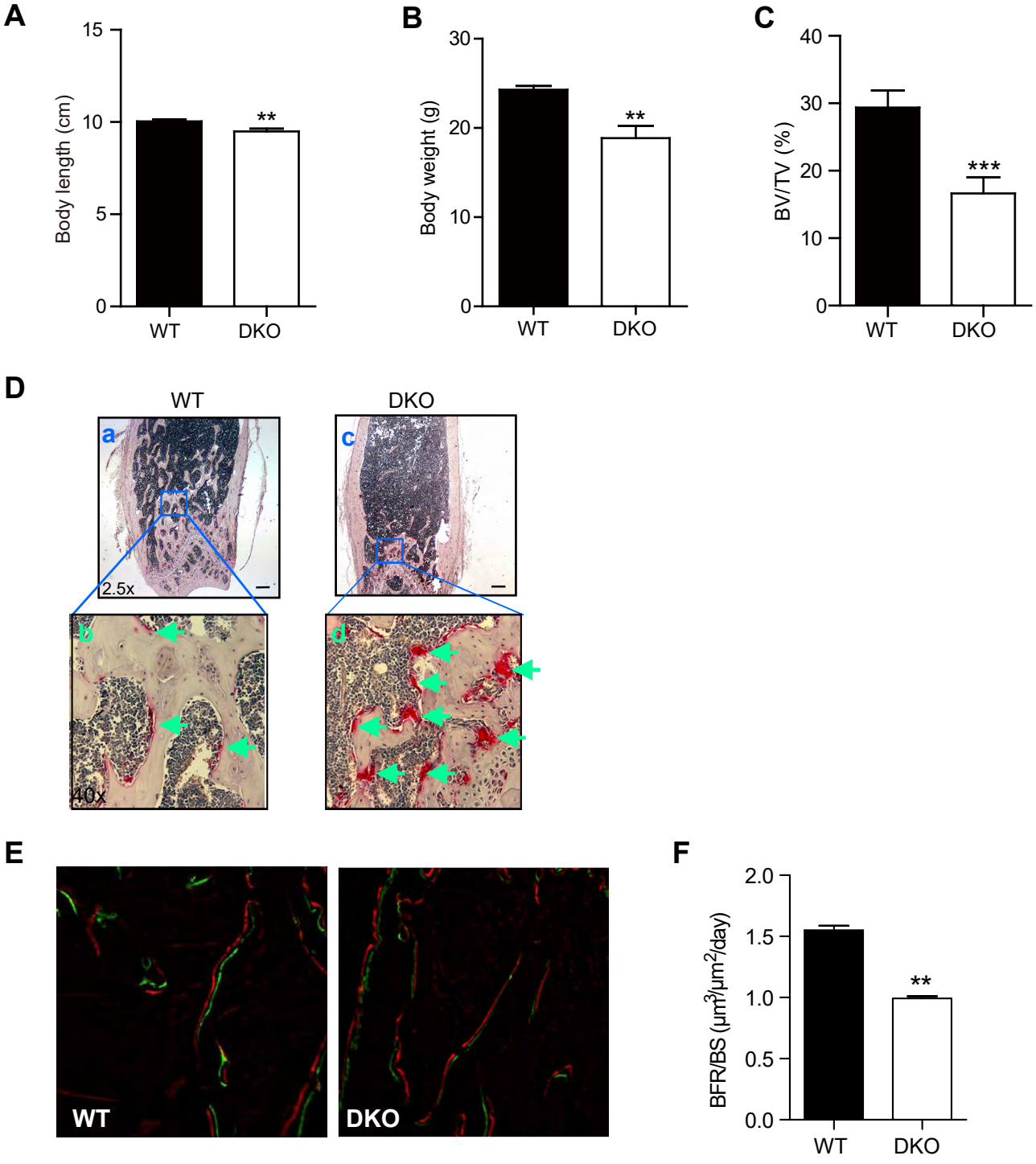




Supplementary Figure 2.

(A, B) DKO mice exhibited short stature (runting, A) and reduced body weight (B) as compared to WT controls (n=20 mice per genotype). Data are presented as mean ± SEM, $^{**}P < 0.01$, 2-tailed Student's *t*-test. **(C)** DKO mice have decreased bone volume as compared to WT mice by quantitative histomorphometric analysis (n=15 mice per genotype). Data are presented as mean ± SEM, $^{***}P < 0.001$, 2-tailed Student's *t*-test. **(D)** Representative photomicrographs at 2.5× and 40× magnification demonstrating TRAP-positive staining osteoclasts (red) lining the trabecular bone surface. **(E)** Representative photomicrographs at 10× magnification demonstrated incorporation of calcein and alizarin red fluorochromes into the trabecular surface during bone remodeling. **(F)** Bone remodeling studies showed that DKO mice had reduced BFR/BS as compared to WT mice (n=3 mice per genotype). Data are presented as mean ± SEM, $^{**}P < 0.01$, 2-tailed Student's *t*-test.

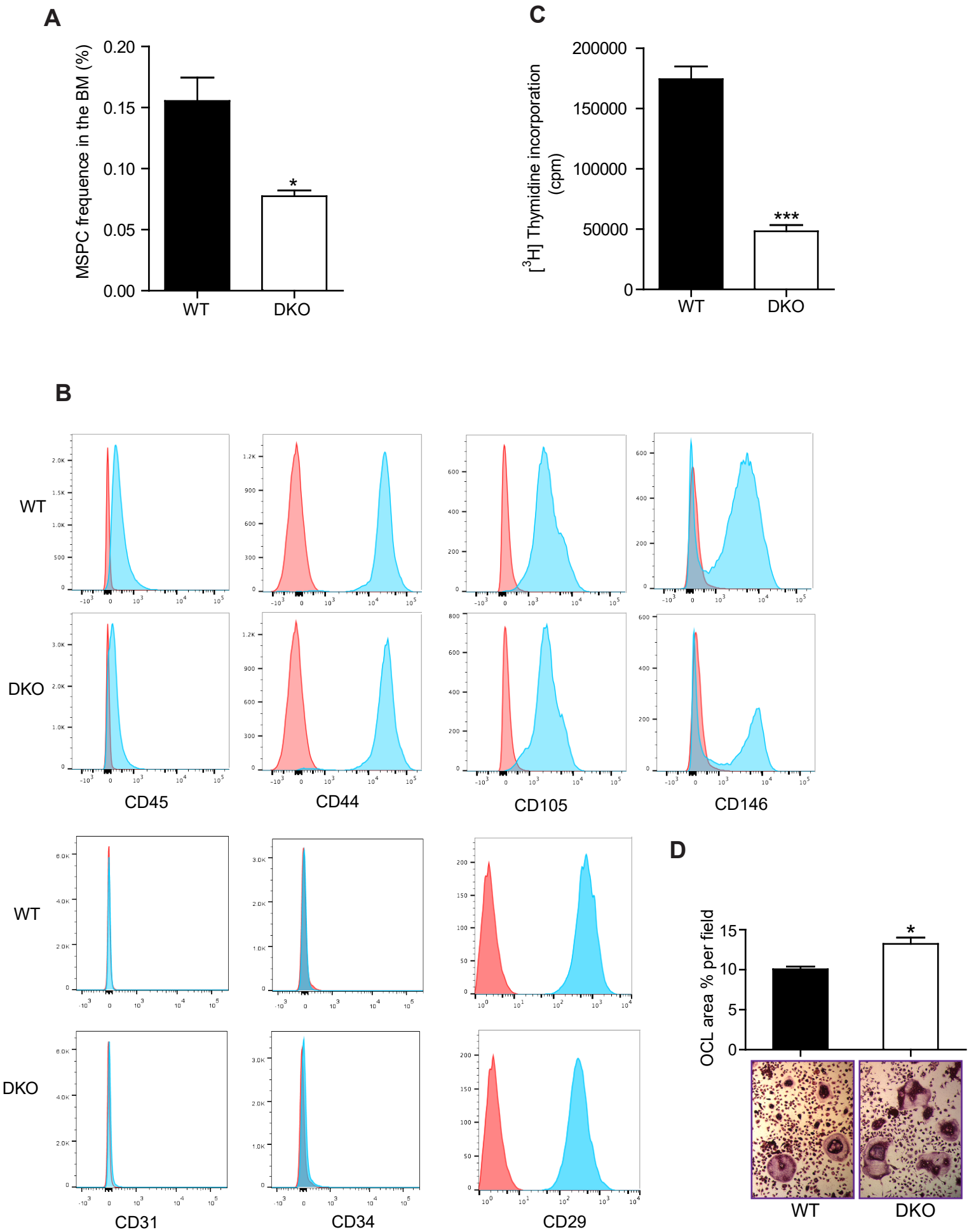
Supplementary Figure 2



Supplementary Figure 3.

(A) The frequency of MSPCs ($CD45^{-}CD146^{+}Nestin^{+}CD105^{+}$) from primary BMMNCs were determined by flow cytometry analysis. Data are presented as mean ± SEM from 3 independent experiments, $*P < 0.05$, 2-tailed Student's *t*-test. **(B)** Representative images of surface marker analysis on MSPCs derived from WT and DKO mice. Isotype controls were shown in red peak, and experimental samples were shown in blue peak. **(C)** Decreased proliferation of DKO MSPCs. Proliferation was measured by a thymidine incorporation assay. Bars represented the mean thymidine incorporation (mean ± SEM) of a representative experiment performed in replicates of 4. $***P < 0.001$, 2-tailed Student's *t*-test. **(D)** DKO mice had enhanced osteoclast (OCL) formation as compared to age- and sex-matched WT mice (n=3 mice per genotype). Data are presented as mean ± SEM, $*P < 0.05$, 2-tailed Student's *t*-test.

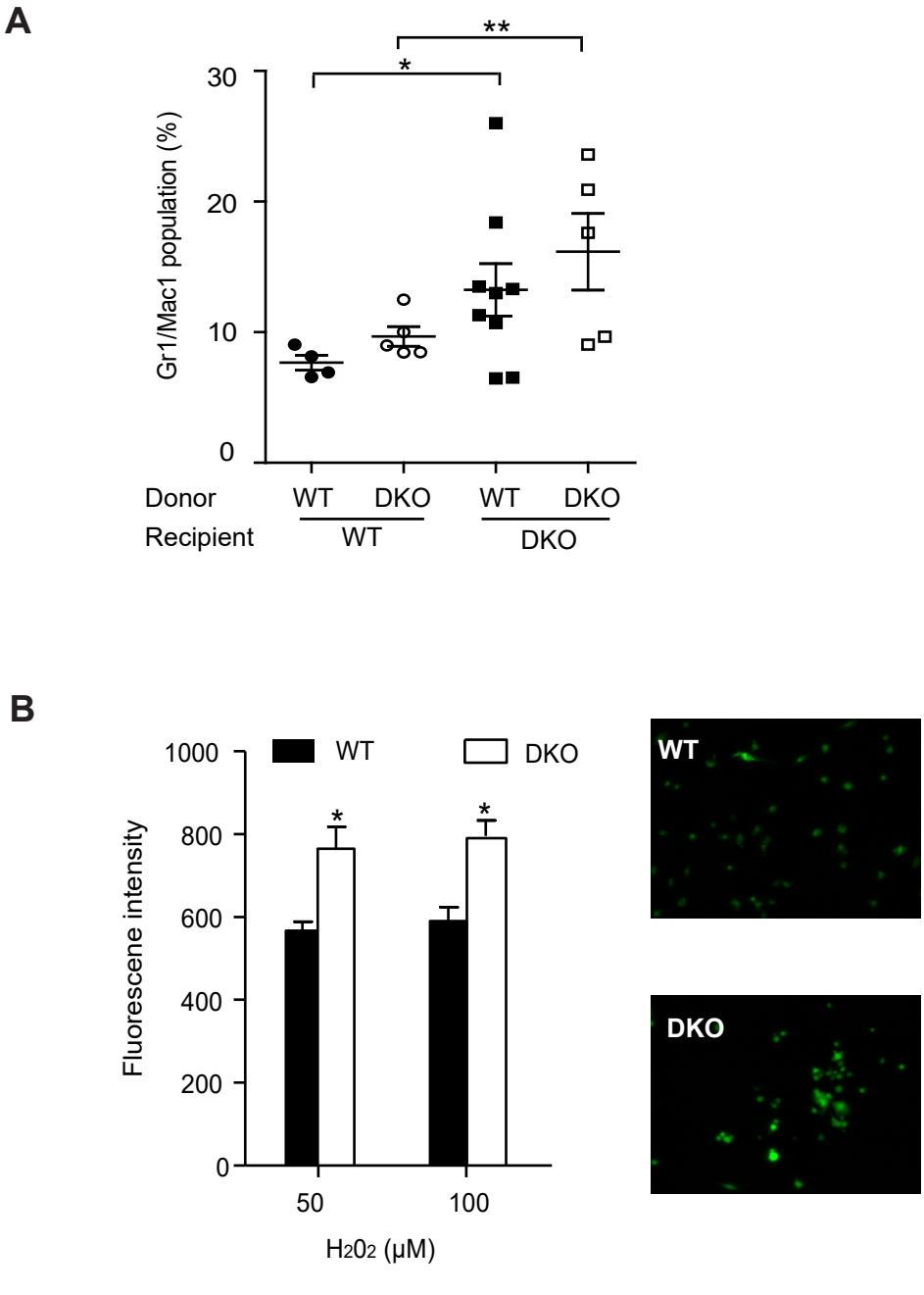
Supplementary Figure 3



Supplementary Figure 4.

(A) WT and DKO recipients have transplanted either WT or DKO bone marrow cells. Nine months later, the mice were sacrificed, and the peripheral blood cells were processed for flow cytometry analysis of Gr1/Mac1-positive myeloid cell population. Data are presented as mean ± SEM, **P* < 0.05, ***P* < 0.01, 2-tailed Student's *t*-test. **(B)** MSPCs from WT and DKO mice were plated in 96-well plate for 24 hours, then exposed with 50 μM and 100μM H_2O_2 in culture media along with the detection reagent and continue culture for 2 hours. Bars graph represented the mean ± SEM of fluorescence intensity from 3 independent experiments. **P < 0.05,* 2-tailed Student's *t*-test. Representative images of immunofluorescent staining of ROS were shown in the right panel.

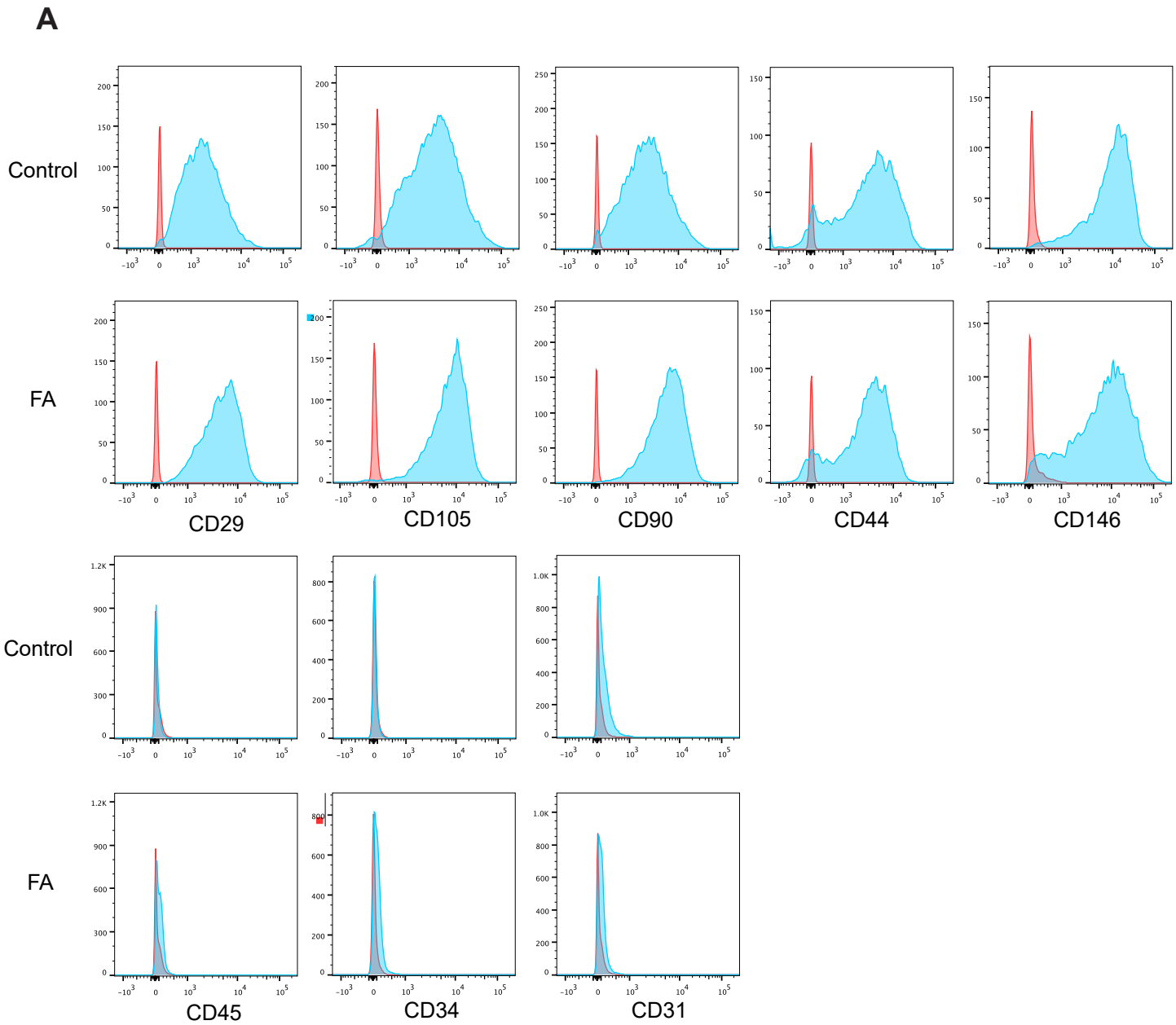
Supplementary Figure 4



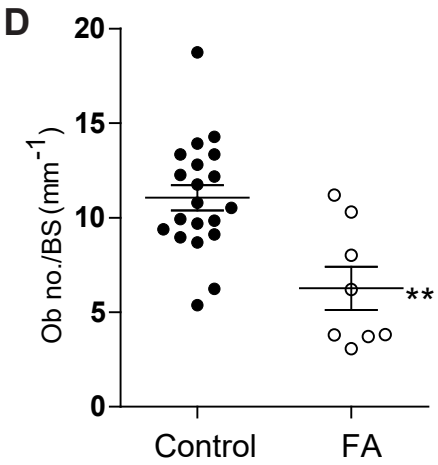
Supplementary Figure 5

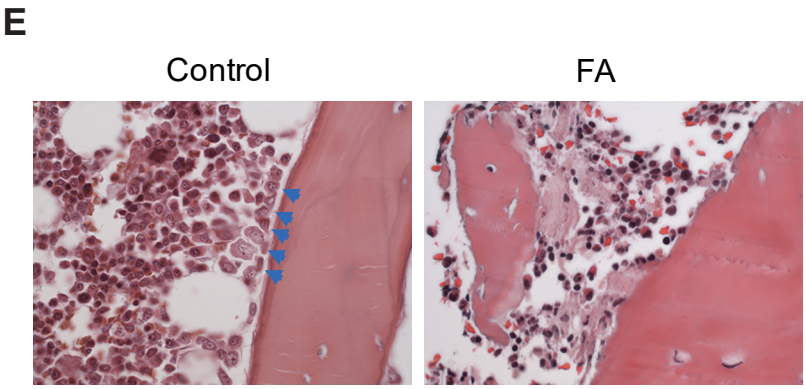
(A) Representative images of surface marker analysis on MSPCs derived from FA patients and a healthy donor. Isotype controls were shown in red peaks, and experimental samples were shown in blue peaks. **(B)** Representative images demonstrated that increased senescent cells were observed in FA MSPCs compared to control MSPCs by β-galactosidase staining. **(C)** Representative images demonstrated that FA patient-derived MSPCs have increased adipocyte differentiative capacity as shown by representative Oil-Red-O staining. Scale bar 50μm**. (D, E)** Reduced osteoblast numbers (Ob no. /BS) in biopsy sections of FA patients compared to that of healthy controls. Data are presented as mean ± SEM, $^{**}P < 0.01$, 2-tailed Student's *t*-test. **(F)** Co-transplantation of normal MSPCs enhanced the engraftment of human FANCG BMMNCs in *NS2* mice while FA MSPCs fail to support FA bone marrow cell expansion.

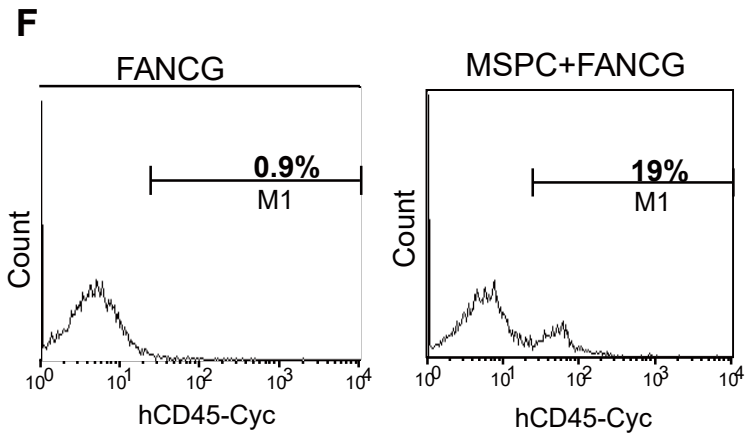
Supplementary Figure 5



Supplementary Figure 5 (Continued)







Supplementary Table 1 Clinical characteristics of FA patients

Patient	Age	Sex	Diagnosis	congenital abnormalities	Hematologic manifestations	MMC-Induced Chromosomal Breakage	Comet Assay	Karyotype
FA1	10	Female	FA	No	progressive pancytopenia	positive	positive (CCP:71%)	46,XX[20]
FA2	6	Male	FA	No	progressive pancytopenia	positive	positive (CCP:69%)	46,XY[20]
FA3	9	Female	FA	Toe syndactyly	progressive pancytopenia	positive	positive (CCP:57%)	46,XX[20]
FA4	4	Male	FA	Hyper and hypopigmentation	progressive pancytopenia	positive	positive (CCP:42%)	46,XY[20]

CCP, comet cell percentage

Supplementary Table 2 Bone remodeling

Genotype	Sex	Lable	dLS	sLS	Thickness	BS	MS/BS	MAR	BFR
DKO	F	5650-1	1151.308	362.809	7.918	2756.308	0.484	1.980	0.957
		5650-2	576.818	53.282	4.874	1370.231	0.440	1.218	0.537
		5650-3	483.879	253.351	8.281	1238.857	0.493	2.070	1.020
		5650-4	777.086	536.501	11.387	2097.526	0.498	2.847	1.419
		5650-5	508.168	473.980	8.368	1608.140	0.463	2.092	0.969
		5650-6	847.291	298.629	10.938	1853.792	0.538	2.734	1.470
		5650-7	1137.915	331.155	7.438	2636.237	0.494	1.860	0.919
		5650-8	402.080	72.190	9.443	793.091	0.552	2.361	1.304
		5650-9	130.448	130.448	10.864	775.750	0.252	2.716	0.685
		5650-10	954.625	101.931	9.471	2954.454	0.340	2.368	0.806
WT	F	5653-1	1410.312	560.260	11.207	3267.456	0.517	2.802	1.449
		5653-2	1078.882	504.937	11.095	2527.329	0.527	2.774	1.461
		5653-3	417.233	170.537	11.663	1251.884	0.401	2.916	1.170
		5653-4	764.101	179.653	12.108	1864.509	0.458	3.027	1.386
		5653-5	1348.336	615.706	11.048	3064.998	0.540	2.762	1.492
		5653-6	1016.487	629.559	9.866	2094.721	0.636	2.467	1.568
		5653-7	1005.062	122.338	10.420	1385.748	0.769	2.605	2.004
		5653-8	1484.661	458.371	12.360	2960.678	0.579	3.090	1.789
		5653-9	737.340	234.237	13.580	1780.163	0.480	3.395	1.630
		5653-10	525.759	30.517	10.249	730.764	0.740	2.562	1.897
DKO	F	5679-1	959.370	1000.827	10.748	2367.633	0.617	2.687	1.657
		5679-2	134.895	186.578	11.527	717.763	0.318	2.882	0.916
		5679-3	141.378	1307.888	9.582	2010.236	0.396	2.396	0.948
		5679-4	81.234	182.844	8.050	469.556	0.368	2.012	0.740
		5679-5	102.471	29.203	7.999	593.784	0.197	2.000	0.394
		5679-6	136.663	20.013	8.570	653.542	0.224	2.143	0.481
		5679-7	154.114	315.298	10.713	920.383	0.339	2.678	0.907
		5679-8	636.793	429.450	8.471	1260.760	0.675	2.118	1.430
		5679-9	1483.539	94.839	12.929	3104.567	0.493	3.232	1.594
		5679-10	215.636	68.716	8.849	1070.861	0.233	2.212	0.516
WT	F	5681-1	831.506	460.049	12.503	2080.252	0.510	3.126	1.595
		5681-2	843.632	212.138	10.340	2000.854	0.475	2.585	1.227
		5681-3	614.694	596.042	11.861	2017.374	0.452	2.965	1.342
		5681-4	576.065	670.574	10.675	1253.002	0.727	2.669	1.941
		5681-5	773.713	238.136	13.868	2158.494	0.414	3.467	1.434
		5681-6	886.286	274.957	10.437	1943.771	0.527	2.609	1.374
		5681-7	517.900	282.800	13.066	750.183	0.879	3.267	2.871
		5681-8	347.555	147.598	9.591	629.445	0.669	2.398	1.605
		5681-9	234.702	187.845	9.474	1522.584	0.216	2.369	0.511
		5681-10	619.673	214.725	11.034	2198.203	0.331	2.758	0.912
DKO	M	5687-1	881.488	830.644	9.110	2843.066	0.456	2.277	1.039
		5687-2	1435.199	1083.670	8.865	4175.695	0.473	2.216	1.049
		5687-3	1255.286	825.386	8.678	3364.897	0.496	2.169	1.075
		5687-4	1581.250	233.930	9.187	3325.596	0.511	2.297	1.173
		5687-5	1667.363	414.518	7.684	3981.270	0.471	1.921	0.905
		5687-6	1601.420	50.794	8.782	3824.409	0.425	2.196	0.934
		5687-7	1671.458	404.774	8.900	3618.838	0.518	2.225	1.152
		5687-8	2060.128	388.622	8.058	4834.748	0.466	2.015	0.939
		5687-9	1141.224	799.792	8.230	3181.079	0.484	2.057	0.997
		5687-10	1142.227	383.037	8.087	3143.343	0.424	2.022	0.858
WT	F	5689-1	1562.975	597.962	11.209	3575.627	0.521	2.802	1.459
		5689-2	2075.718	1057.627	11.040	4669.020	0.558	2.760	1.540
		5689-3	1780.977	396.679	8.772	2821.441	0.702	2.193	1.538
		5689-4	1646.492	229.430	9.942	2058.197	0.856	2.486	2.127
		5689-5	1679.064	448.783	9.635	2950.466	0.645	2.409	1.554
		5689-6	1825.499	847.329	11.954	3329.591	0.676	2.989	2.019
		5689-7	357.462	215.717	5.326	689.148	0.675	1.331	0.899
		5689-9	2060.729	620.759	10.916	2812.181	0.843	2.729	2.301
		5689-10	309.642	188.057	11.782	1085.679	0.372	2.946	1.095

MAR (mineral apposition rate)=Thickness/time

MS/BS (mineralizing surface per bone surface)=(dLS+sLS/2)/BS

BFR(bone formation rate)/BS=MAR*(MS/BS)

Supplemental Methods

Reciprocal transplantation

Six- to eight-week-old WT or *DKO* mice were irradiated with 1100 cGy. Four hours after irradiation, the mice were anesthetized with Avertin at 375 μg/g body weight. A total of 10^6 BMMNCs in 100-μL volume was injected into the tail vein of the WT and *DKO* mice.

BMD quantification

Bone mineral density (BMD) was measured using peripheral dual-energy X-ray absorptiometry (pDEXA) with a Lunar Piximus densitometer (GE Medical Systems, software version 1.4 Lunar).[1] The mice were anesthetized with avertin/tribromoethanol (0.25mg/kg) and placed into the scanner in the prone position with arms and legs extended. The BMD of the left femoral metaphysis was measured by defining a region of interest of 11 x10 pixels proximal to the distal growth plate, a region with a high content of trabecular bone.

Clonogenic Assays

CFU-C in the bone marrow were assayed as previously described.[2] Briefly, 5 x 10^4 BMMNCs were seeded in a 35-mm gridded dish containing methylcellulose and murine recombinant granulocyte-macrophage colony-stimulating factor (GM-CSF), murine interleukin 3 (IL-3), murine stem cell factor (SCF) (Rocky Hill, NJ), M-CSF (R&D Research Laboratories; Minneapolis, MN), and cultured at 37°C in a 5% CO_2 incubator for 7 days. To

measure the frequency of MSPCs in bone marrow, the CFU-F assay was performed as previously reported.[3] Briefly, BMMNCs were plated at 2 x 10^6/mL into 6-well tissue culture plates in triplicate for each condition in complete MesenCult medium (MesenCult basal media plus 20% of MesenCult Supplemental, Stem Cell Technologies Inc., Vancouver, Canada) and incubated at 37°C, 5% CO_2. After 14 days of culture, the medium was removed, and each well was washed with phosphate-buffered saline, stained with HEMA-3 quick staining kit (Fisher Scientific Company, VA, USA) according to the manufacturer's instructions and photographed. A Fuji film digital camera (FinePix2400Zoom, Fuji Photo Film Co., Japan) was used to acquire the photomicrographs of the CFU-F.

Purification of HSPCs

HSPCs were purified as described previously.[4] Briefly, mature cell lineage antigen-negative (Lin^-) cells were enriched by magnetic-activated cell sorting (MACS; Miltenyi Biotech, Auburn, CA), with the use of a mixture of purified rat anti-mouse mAbs specific for the mature cell lineage antigens CD45R (B220, Clone RA3-6B2), Gr1 (Ly-6G, Clone RB6-8C5), CD4 (L3T4, Clone RM4-5), CD8a (Ly-2, Clone 53-6.7), TER119 (Ly-76, Clone TER119), and Mac1 (CD11b, Clone M1/70) (BD-Pharmingen, San Diego CA). The nonmagnetic Lin^- fraction was collected, washed, and counted. The cells were then incubated with rat anti-mouse CD32/CD16 to avoid nonspecific antibody binding, after which they were stained with fluorescein isothiocyanate (FITC) labeled rat anti-mouse

CD117 (c-Kit) (Clone 2B8, BD-Pharmingen). Negative control cells were stained with phycoerythrin-conjugated IgG2a and FITC-conjugated IgG2b. Based on these controls, $Lin^-c\text{-}Kit^+$ cells were isolated by sorting with a fluorescence-activated cell sorter (FACStar Plus; Becton Dickinson) under sterile conditions. The purity of $Lin^-c\text{-}Kit^+$ cells thus obtained was >90% (data not shown).

Long-term culture of HSPCs on MSPC monolayers

To evaluate the effect of MSPCs in supporting proliferative hematopoietic cells, long-term co-culture of MSPCs with $Lin^-c^-Kit^+$ hematopoietic progenitors was performed as previously described.[5] Briefly, adherent MSPC layers were obtained by seeding with 1.5 x 10^5 MSPCs/well in 1 mL IMDM containing 10% horse serum, 10% FBS, 5 x 10^{-7} M hydrocortisone in a 24-well culture plate. The cultures were maintained at 33°C in a 5% CO_2 incubator. When the MSPC monolayer reached 90% confluence, the cultures were irradiated at 15 Gy and used as supporting cells. To initiate long-term cultures, the medium was completely removed from the irradiated MSPC cultures and replaced with fresh medium. $Lin^-c\text{-}Kit^+$ cells (5 x 10^4) were inoculated on the irradiated cell layers in triplicate wells per condition and incubated at 33°C in a 5% CO_2 incubator. Following four weeks of weekly half-medium changes, the supportive effect of MSPCs to HSPCs was evaluated by counting cobblestone area-forming cells (CAFCs) as described previously.[6,7]

AnnexinV/ propidium iodide (PI) staining assay

To evaluate whether loss of both *Fancc* and *Fancg* in MSPCs alters cell apoptosis, the percentage of Annexin-V-FITC /propidium iodide (PI) positive cells after serum deprivation was evaluated by flow cytometry as described previously.[8] Briefly, when MSPC cultures reached 70-80% confluence, the cultures were maintained in DMEM without serum for 24 hours. The cells were detached from the culture plate with 0.05% Trypsin-EDTA, harvested and then resuspended in 100 µL of binding buffer (10 mM HEPES/NaOH [pH 7.4], 140 mM NaCl, 2.5 mM $CaCl_2$). Cells were then stained with 5 µL of Annexin V-FITC (BD-Pharmingen) and 500 ng of PI (Calbiochem, La Jolla, CA), incubated at room temperature for 15 minutes in the dark and analyzed by flow cytometry.

Senescence Assay

Histochemical staining for β-galactosidase activity was utilized to determine the MSPC senescence.[3,9] WT and DKO MSPCs were plated in chamber slides at 2 x 10^4/chamber and incubated at 37°C, 5% CO_2 for 72 hours. Cells were then stained with a Senescence Staining Kit (Sigma, USA) according to the manufacturer's instructions. Senescent cells displayed a blue color in the cytoplasm. A total of 2000 cells were counted in five random fields per well to determine the percentage of SA-β-gal positive cells.

qPCR

Total RNA was extracted with TRIzol reagent (Invitrogen). Reverse

transcription-PCR amplifications were performed using M-MLV (Invitrogen) according to the manufacturer's instructions. qPCR was performed using SYBR Green chemistry (Thermo Scientific, MA, USA) on an ABI Prism 7500 detection system. *Gapdh* was used as the reference gene, and expression differences were calculated.

Thymidine incorporation assay

To examine the in vitro proliferative capacity, 10^4 MSPCs from DKO and WT mice were plated in 96-well plates in 200 μL α-MEM without supplements for 24hours. The cells were then cultured for another 48 hours in complete medium with MesenCult supplements, then pulsed with 1μCi [^{3}H] thymidine (Amersham Pharmacia Biotech) 6 hours before harvest. γ emission was measured using a Beckman Coulter LS 6500 Scintillation Counter.

ROS detection

The MSPCs from WT and DKO mice were plated in 96-well plate at a density of 5000/well on the day for the experiment. Twenty-four hours after seeding, MSPCs were exposed with 50uM and 100uM H_2O_2 in culture media along with the detection reagent and continue culture for 2 hours.The cells were then washed with wash buffer and processed for imaging according to manufacturer’s protocol (ROS detection kit, ENZ-51011 from Enzo).

Osteoblast and adipocyte differentiation

For osteoblast differentiation, 5 x 10^4 MSPCs were cultured for seven days in 6-well plates using osteogenic differentiation medium (MSC medium supplemented with 10^{-7} M dexamethasone, 50 µg/mL ascorbic acid and 10 mM β-glycerophosphate) as previously described [10]. To determine the ALP activity, cells were fixed in citrate-buffered acetone for 30 seconds, incubated in the alkaline-dye mix for 30 min and counter-stained with Mayer's Hematoxylin for 10 min. Cells were then evaluated microscopically, and ALP^+ area was recorded. To induce adipocyte differentiation *in vitro*, 1 x 10^5 MSPCs were plated in 6-well tissue culture plates for 14 days and cultured with adipogenic differentiation medium (MSC medium supplemented with 10^{-7} M dexamethasone, 450 µM 3-isobutyl-1- methylxanthine, 1 µg/ mL insulin and 200 µM indomethacin) as previously described.[10] Adipocyte differentiation was quantified by Oil-red O staining on day 14 of culture.[11]

Co-transplantation of FA BMMNCs and MSPCs in NS2 mice

MSPCs derived from a healthy donor were intra-tibial injected into sub-lethally irradiated NS2 recipient mice (10^6/mouse). Twenty-four hours after MSPC injection, BMMNCs (3x10^6/mouse) from a human *FANCG* deficient patient were delivered via tail vein injection. Four months following co-transplantation, $hCD45^+$ cell engraftment within the bone marrow of recipient mice was analyzed by flow cytometry.

Reference

1. Yang FC, Chen S, Robling AG, et al. Hyperactivation of p21 and PI3K cooperate to alter murine and human neurofibromatosis type 1-haploinsufficient osteoclast functions. J Clin Invest. 2006;116(11):2880-2891.
2. Yang FC, Watanabe S, Tsuji K, et al. Human granulocyte colony-stimulating factor (G-CSF) stimulates the in vitro and in vivo development but not commitment of primitive multipotential progenitors from transgenic mice expressing the human G-CSF receptor. Blood. 1998;92(12):4632-4640.
3. Wu X, Estwick SA, Chen S, et al. Neurofibromin plays a critical role in modulating osteoblast differentiation of mesenchymal stem/progenitor cells. Hum Mol Genet. 2006;15(19):2837-2845.
4. Yang FC, Atkinson SJ, Gu Y, et al. Rac and Cdc42 GTPases control hematopoietic stem cell shape, adhesion, migration, and mobilization. Proc Natl Acad Sci U S A. 2001;98(10):5614-5618.
5. Li Y, Chen S, Yuan J, et al. Mesenchymal stem/progenitor cells promote the reconstitution of exogenous hematopoietic stem cells in Fancg-/- mice in vivo. Blood. 2009;113(10):2342-2351.
6. Terpstra W, Rozemuller H, Breems DA, et al. Diphtheria toxin fused to granulocyte-macrophage colony-stimulating factor eliminates acute myeloid leukemia cells with the potential to initiate leukemia in immunodeficient mice, but spares normal hemopoietic stem cells. Blood. 1997;90(9):3735-3742.
7. Issaad C, Croisille L, Katz A, Vainchenker W, Coulombel L. A murine stromal cell line allows the proliferation of very primitive human CD34++/CD38- progenitor cells in long-term cultures and semisolid assays. Blood. 1993;81(11):2916-2924.
8. Yang FC, Kapur R, King AJ, et al. Rac2 stimulates Akt activation affecting BAD/Bcl-XL expression while mediating survival and actin function in primary mast cells. Immunity. 2000;12(5):557-568.
9. Michaloglou C, Vredeveld LC, Soengas MS, et al. BRAFE600-associated senescence-like cell cycle arrest of human naevi. Nature. 2005;436(7051):720-724.
10. Zhang P, Xing C, Rhodes SD, et al. Loss of Asxl1 Alters Self-Renewal and Cell Fate of Bone Marrow Stromal Cell, Leading to Bohring-Opitz-like Syndrome in Mice. Stem Cell Reports. 2016;6(6):914-925.
11. Xu MJ, Tsuji K, Ueda T, et al. Stimulation of mouse and human primitive hematopoiesis by murine embryonic aorta-gonad-mesonephros-derived stromal cell lines. Blood. 1998;92(6):2032-2040.