

SUPPLEMENTAL TABLES

Table S1: List of antibodies used for flow cytometry related to the main Figures 2, 4 and 5, and SF1 and 2.

Anti-mouse Antibody	clone	Isotype	Supplier
CD31	MEC 13.3	rat IgG2a	BD Pharmingen
CD34	RAM34	rat IgG2a	Invitrogen
CD45	30F11	rat IgG2b	Sony
CD45.1	A20	mouse IgG2a	Sony
CD45.2	104	mouse IgG2a	Biolegend
CD48	HM48-1	Armenian hamster IgG	BD Pharmingen
CD51	RMV-7	rat IgG1	Sony
CD71	C2	rat IgG1	BD Pharmingen
CD117	2B8	rat IgG2b	Sony
CD135	A2F10	rat IgG2a	Biolegend
CD140a	APA5	rat IgG2a	BD Horizon
CD150	TC15-23F12,2	rat IgG2a	Sony
Cxcr4	clone 2B11	rat IgG2b	BD Biosciences
Ackr3	clone 8F11-M16	mouse IgG2b	BioLegend
KI67	B56	mouse IgG1	BD Biosciences
Sca-1	E13-161.7	Rat IgG2a	Sony
Ter119	TER-119	rat IgG2b	Sony
Anti-human Antibody	clone	Isotype	Supplier
CD45	J33	mouse IgG1	BD Biosciences
CD73	AD2	mouse IgG1	BD Biosciences
CD90	F15-42-1-5	mouse IgG1	BioLegend
CD105	1G2	mouse IgG3	BD Biosciences
CXCR4	12G5	mouse IgG2a	BD Biosciences
ACKR3/CXCR7	8F11-M16	mouse IgG2b	BioLegend

Anti-mouse Lin cocktail: anti-CD3, anti-CD45R, anti-CD11b, anti-TER119, anti-CD41 and anti-Gr-1 mAbs (BD Biosciences)

Table S2: List of antibodies used for immunofluorescence related to the main Figures 1, 2, 4 and 5, and SF2.

Origin	Antibody	Conjugate	Supplier
Mouse	Cxcl12	Purified	R&D
pGoat	Opn	Purified	R&D
pGuinea pig	Perilipin A	Purified	Research Diagnostic Inc
pRabbit	Osterix	purified	Santa Cruz
Mouse	IgG1	Purified	Invitrogen
pGoat	Mouse	AF633	Invitrogen
pRabbit	Guinea pig	TRITC	OriGene Technologies
pDonkey	rabbit	Dylight 550	Thermofisher

Table S3: List of primers used for quantitative PCR related to the main Figures 3, 4, 5, and 6 and SF1.

Genes	Forward 5'→3'	Reverse 5'→3'
Mouse (m) and human (h) osteogenic differentiation		
<i>mOcn</i>	GGGCAATAAGGTAGTGAACAG	GCAGCACAGGTCCTAAATAGT
<i>hOCN</i>	CACCGAGACACCATGAGAGC	CTGGGTCTCTTCACTACCTC
<i>mAlp</i>	CACAATATCAAGGATATCGACGTGA	ACATCAGTTCTGTTCTTCGGGTACA
<i>mOsx</i>	ATGGCGTCCTCTCTGCTTGA	GAAGGGTGGGTAGTCATTTG
<i>hOSX</i>	TGCTTGAGGAGGAAGTTCAC	AGGTCACTGCCCACAGAGTA
<i>mOpn</i>	GCCTGTTTGGCATTGCCTCCTC	CACAGCATTCTGTGGCGCAAGG
<i>hOPN</i>	TCTAAGAAGTTTCGCAGACC	ATGTCCTCGTCTGTAGCATC
<i>mRunx2</i>	ACGAGGCAAGAGTTTCACC	GGACCGTCCACTGTCACTTT
<i>hRUNX2</i>	AGTGGACGAGGCAAGAGTTTCA	GGGTTCCCGAGGTCCATCTA
Mouse (m) chondrogenic differentiation		
<i>mSox9</i>	TACGACTGGACGCTGGTGCC	CCGTTCTTCACCGACTTCCTCC
<i>mAggrecan</i>	GCCTCTCAAGCCCTTGTCTG	CACCCCTCCTCACATTGCTC
<i>mCol2α1</i>	CTGACCTGACCTGATGATACC	CACCAGATAGTTCTGTCTCC
Mouse (m) adipogenic differentiation		
<i>mPparγ</i>	GACCACTCGCATTCTTTT	CCACAGACTCGGCACTCA
<i>mFabp4</i>	CTTGTGGAAGTCACGCCTTT	AAGAGAAAACGAGATGGTGACAA
<i>mPlnl</i>	AGCGTGGAGAGTAAGGATGTC	CTTCTGGAAGCACTCACAGG
Mouse (m) MSC markers		
<i>mCd51</i>	ACCACTAACATCACCTGGGG	TCTTCTTGAGGTGGTCGGAC
<i>mSca-1</i>	GCTGATTCTTCTTGTGGCCC	CCACAATAACTGCTGCCTCC
<i>mPdgrα</i>	CGACTGGATGATCTGCAAGC	GCTGAGGTTGTTCTTTGCCA
Mouse (m) osteoclastogenic differentiation		
<i>mTnfsf11</i>	GGAAGTCAACACATTGTGGG	GCCTTCCATCATAGCTGGAGC
<i>mTnfrs11b</i>	TCCGGCGTGGTGCAA	AGAACCCATCTGGACATTTTTTG
<i>mCsfl</i>	TTAAAGACAACACCCCCAATGC	TCAGGTTATTGGAGAGTTCCTGGA
Mouse (m) osteoclastic differentiation		
<i>mNfatc1</i>	TGAGGCTGGTCTTCCGAGTT	CGCTGGGAACACTCGATAGG
<i>mCln7</i>	CTTGAAGCATAAGGTGTTTGTGGA	CTCAGTCGCCGCTGCAC
<i>mCtsk</i>	CAGCAGAGGTGTGTACTATG	GCGTTGTTCTTATTCCGAGC
<i>mTnfrs11a</i>	CTTGGACACCTGGAATGAAGAAG	AGGGCCTTGCCTGCATC
Mouse (m) and human (h) housekeeping genes		
<i>mActin</i>	GGGTCAGAAGGACTCCTATG	GGTCTCAAACATGATCTGGG
<i>hACTIN</i>	AGTCATTCCAAATATGAGATGCGTT	TGCTATCACCTCCCCTGTGT
<i>m36b4</i>	TCCAGGCTTTGGGCATCA	CTTTATCAGCTGCACATCACTCAGA
<i>hGAPDH</i>	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG

Table S4: List of primers used for the BioMark assay related to the main Figure 4.

Genes	Primers
Osteogenic markers	
<i>Collα</i>	Mm00801666_g1
<i>Alp</i>	Mm00475834_m1
<i>Ibsp</i>	Mm00492555_m1
<i>Runx2</i>	Mm00501584_m1
Cell cycle	
<i>Ccnd2</i>	Mm00438070_m1
<i>Ccnd3</i>	Mm01612362_m1
Irrelevant genes (negative controls)	
<i>Pax5</i>	Mm00435501_m1
<i>CD3e</i>	Mm00599684_g1
Housekeeping gene	
<i>Actinβ</i>	Mm01205647_g1

SUPPLEMENTAL FIGURES

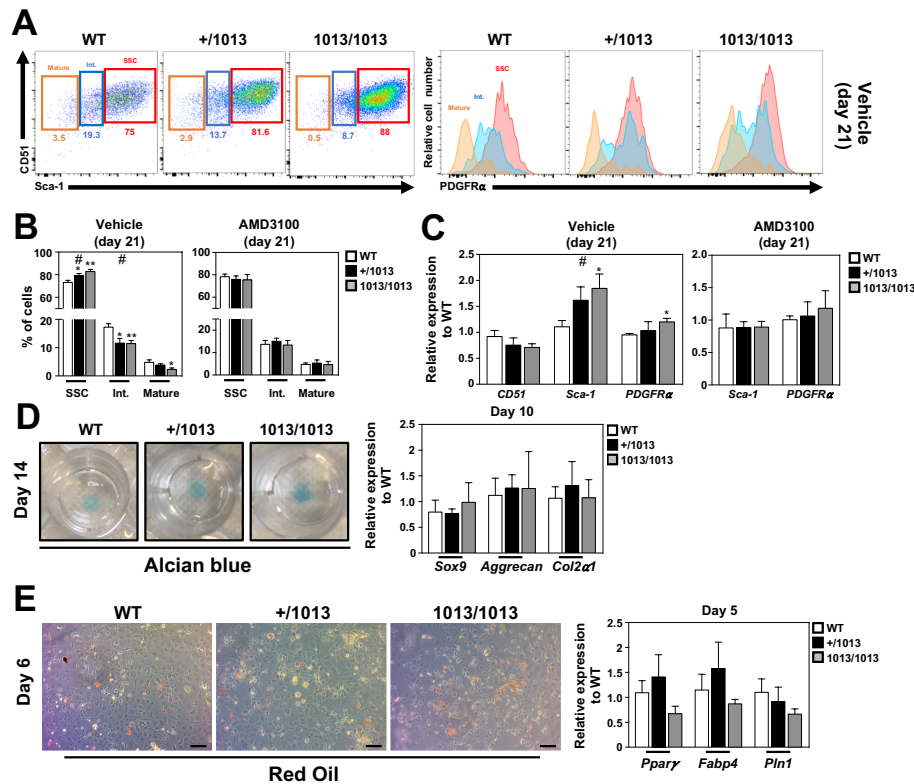


Figure S1 related to Figure 5: (A and B) Representative flow-cytometric analyses based on CD51, Sca-1 and PDGFR α surface markers (A) and the frequencies (means $\pm$ SEM) (B) of SSCs and cells with intermediate or most mature phenotype within vehicle- and AMD3100-treated WT and mutant cultures at day 21. **(C)** Expression levels of stromal genes (*CD51*, *Sca-1* and *PDGFR α*) were determined by qRT-PCR in SSC cultures after 21 days of osteogenic culture. Each individual sample was run in triplicate and was standardized for β -actin expression levels. Results (means $\pm$ SEM) are expressed as relative expression to WT samples. **(D)** Left: Alcian blue staining was performed 14 days after initiation of the culture of WT and mutant SSCs in chondrogenic medium. Right: Expression levels of chondrogenic genes (*Sox9*, *Aggrecan* and *Col2 α 1*) were determined by qRT-PCR in WT and mutant cultures at day 10. Each individual sample was run in triplicate and has been standardized for *36b4* expression levels. Results (means $\pm$ SEM) are expressed as relative expression to WT samples and are

from 3 independent SSC cultures per genotype. **(E)** Left: Oil Red O staining was performed 6 days after initiation of cultures of WT and mutant SSCs in adipogenic medium. Right: Expression levels of adipogenic genes (*Pparγ*, *Fabp4* and *Pln1*) were determined by qRT-PCR in WT and mutant cultures at day 5. Each individual sample was run in triplicate and has been standardized for *36b4* expression levels. Results (means $\pm$ SEM) are expressed as relative expression to WT samples and are from 3 independent MSC cultures per genotype. Kruskal–Wallis *H* test–associated p-values (#) are indicated. *, $P < 0.05$ and **, $P < 0.005$ compared with WT samples (as determined using the two-tailed Student's *t* test).

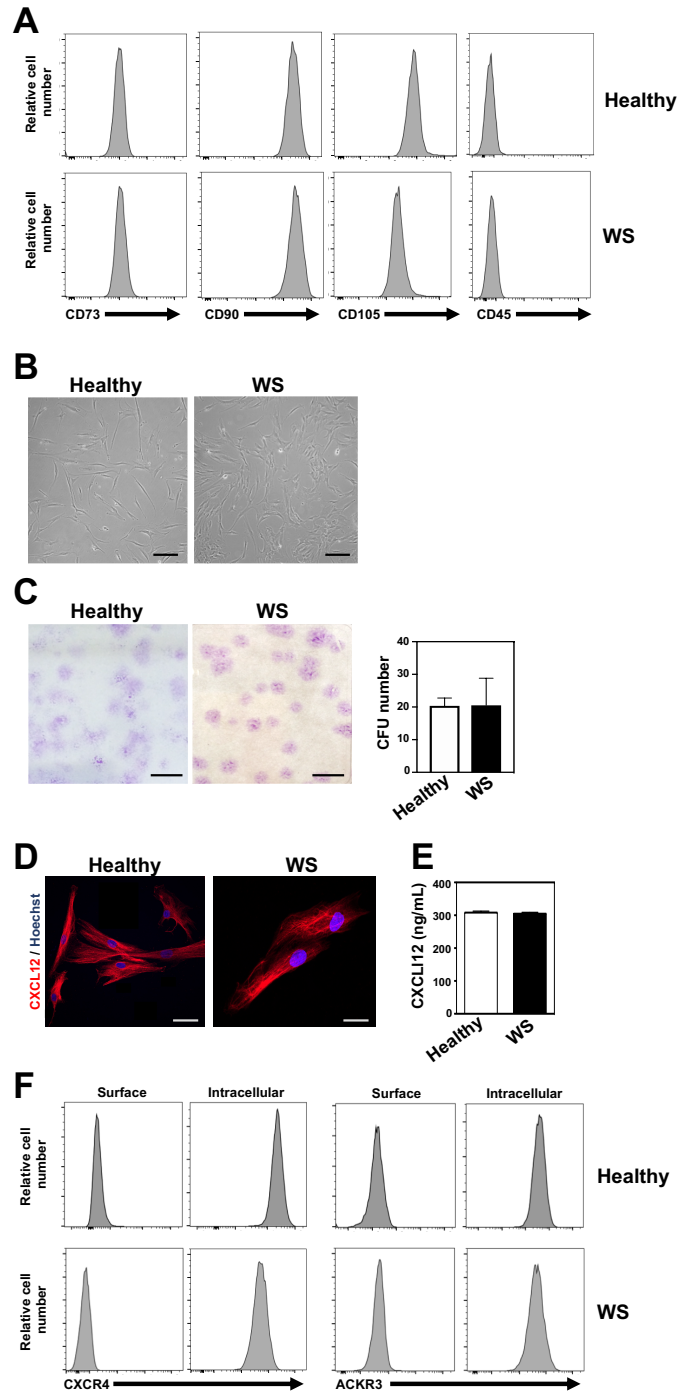


Figure S2 related to Figure 6:

(A) Surface detection of CD73, CD90 and CD105 was determined by flow cytometry on *in vitro* expanded BMSCs from a representative healthy donor (top) or WS patient (bottom). BMSCs were negative for the hematopoietic marker CD45. (B) Bright field pictures of the corresponding primary healthy and WS BMSC cultures. Healthy and WS BMSCs were all spindle shaped and fibroblast-like cells. At all culture passages, WS MSCs exhibited a

fibroblast-like morphology. Bars: 200 μm . Pictures are representative of 7 healthy and 2 WS BMSC cultures. **(C)** Representative crystal violet staining of colonies formed from 0.2×10^3 BMSCs of healthy or WS donors (left). Bars: 1 cm. Quantification of the number of colonies (means $\pm$ SEM) obtained in these CFU-F assays, each individual sample having been run in triplicate (right). **(D and E)** CXCL12 expression were determined in BMSC cultures by immunofluorescence and ELISA. Immunofluorescence staining of CXCL12 in association with Hoechst 33342 in healthy and WS BMSC cultures is shown (D, bars: 50 μm). Images are representative of five independent determinations. CXCL12 secreted in the supernatants from BMSC cultures of healthy and WS donors were determined by ELISA (E). Data (means $\pm$ SEM) are from two independent cultures. **(F)** Expression levels of CXCR4 (left) or ACKR3 (right) were determined by flow cytometry in BMSCs from healthy and WS donors before (surface) and after (intracellular) permeabilization. Representative histograms for surface or intracellular detection of CXCR4 or ACKR3 are shown.