SUPPLEMENTARY INFORMATION

1. Supplementary Experimental Procedures: 9 sections
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SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Animals and Arthritis Induction

C57BL/6 (B6; Ly5.2) and B6.SJL-Ptprc^{a}Pep3^{b}/BoyJ (B6; Ly5.1) were purchased from Jackson Laboratory and maintained in our suite. KRN TCR transgenic mice on a B6 background (KRN; Ly5.2) and C57BL/6 congenic mice bearing arthritogenic I-A$^{g7/g7}$ MHC-II molecule (G7; Ly5.2) [1] were both obtained from Dr. Paul Allen (Washington University) and maintained in our suite.

The G7;Ly5.1 strain was generated in this study from G7;Ly5.2 and B6;Ly5.1 mice first to generate B6xG7 (Ly5.1xLy5.2) and then intercrossing F1s to generate G7;Ly5.1. G7 genotyping was performed using a biotin conjugated anti – G7 antibody kindly provided by Dr. Emil Unanue (Washington University). B6xG7 mice were generated from B6;Ly5.2 or B6;Ly5.1 crossed with G7;Ly5.2 or G7;Ly5.1 parents to get mice with different *Ly5* alleles - Ly5.1+Ly5.2-, Ly5.1+Ly5.2+, Ly5.1-Ly5.2+. KRNxG7 were generated by crossing KRN mice with G7;Ly5.2 or G7;Ly5.1 mice to get arthritic mice with different *Ly5* alleles – Ly5.1-Ly5.2+ and

Ly5.1+Ly5.2+ respectively. In all experiments, KRN, G7 and/or B6xG7 mice, which are all non-arthritic were used as controls to arthritic KRNxG7 mice.

For serum transfer arthritis, mice 7 days post serum transfer were used for experiments. Development of arthritis was confirmed visually and by measurement of ankle thickness since arthritis development was variable and not as robust as KRNXG7 spontaneous chronic model consistent with previous reports [2].

Tissue Preparation

Where analyzed, peripheral blood (PB) was collected by retroorbital bleeding before sacrificing mice. Mice were sacrificed by cervical dislocation or CO_2 asphyxiation. Bone marrow (BM) was collected from tibia(e) and/or femur(s) by flushing as previously described [3] or by brief high speed centrifugation of bones longitudinally aligned in a perforated eppendorf tube. Retrieved cells were red blood cell (RBC) lysed (RBC lysis buffer, Roche) or prepared for magnetic sorting without RBC lysis as described below. Peripheral blood was subjected to 2 rounds of RBC lysis due to abundant red blood cells.

Flow Cytometry and Fluorescence Activated Cell Sorting (FACS)

Antibodies used in this study are listed in Table S1. Flow cytometry was performed on a FACScalibur (BD) and modified FACScans (BD) with 2 and 3 lasers capable of 5 and 8 color analyses respectively or on a MoFlo (Dako). Data was analyzed using FlowJo software (Treestar).

For purification of GMP, KSL or HSC enriched fractions of KSL cells harvested whole bone marrow cells were initially depleted of mature cells using MACS lineage depletion kit (Miltenyi) and LS columns (Miltenyi) prior to FACS. Specifically, bone marrow from both tibiae and femurs (about $50x10^6$ cells) were incubated with 40μl of lineage antibody cocktail followed by 80μl of microbead conjugated anti-biotin antibody. Magnetic sorting does not completely deplete Lin+ cells especially for arthritic mice, probably because the Lineage antibody cocktail formulation is tailored to lineage frequencies present in control mice which are different from those in arthritic mice. Therefore, we stained lineage-depleted bone marrow with fluorophore conjugated lineage marker antibodies to gate out residual Lin+ cells during FACS. These cells were also stained with fluorophore conjugated progenitor marker antibodies: α-Kit, α-Sca1, α-FcgR. KSL cells or GMPs were sorted using MoFlo (Dako). Purity of KSL sorting was about 95% determined by post sort analysis. Lineage antibodies used were α-Ter119 (for erythrocytes), α-B220 (for B cells), α-Mac1 and α-Gr1 (for myeloid cells) and α-CD3, α-CD4, α-CD8 and/or α-TCRβ (for T cells). Common lymphoid progenitors were excluded by restrictive gating on Kit^{hi} cells and by addition of α-IL7Rα to the lineage cocktail.

For Ki67 intracellular staining of HSC enriched populations, BM cells were lineage depleted by magnetic sorting (as described above). Lineage depleted cells were surface stained with antibodies to Kit, Sca1, lineage markers and IL7Rα, as described above, as well as CD150 and CD48. PerCP-Cy5.5 conjugated IL7Rα, CD48, and lineage antibodies were used to minimize the number of channels for analysis. Cells were then fixed and permeabilized using BD cytofix/cytoperm solution. Fixed cells were then incubated with Ki67 FITC antibody (BD) or FITC conjugated isotype control antibody (BD).

Methylcellulose Colony Replating

KSL cells or GMP enriched cells (Kit+Sca1-Lin-IL7Rα-FcγRIII/II^{hi}) from 6-9 week old, age and sex-matched (male) arthritic and control mice were sorted on a MoFlo as described above. KRN, G7 or B6xG7 mice were used as controls. Granulocyte and Macrophage (GM) restricted (HSC 008; R & D) and Erythroid restricted (Methocult SF M3436; Stem Cell Technologies) methylcellulose based media were used for replating according to manufacturer's product information sheet with minor modifications. Each population (KSL or GMP) from each mouse was plated in duplicate. 200 cells per plate were used for replating on GM-restricted plates. KSL cells formed much fewer colonies in erythroid restricted plates and so 1000 cells per plate was used. GM colonies were enumerated on day 7-10 while erythroid colonies were enumerated on D14. Colony counts were performed in a blinded manner and mean count of duplicate plates for each mouse determined. These values were then used in subsequent statistical analysis. Plating efficiency was calculated as (number of colonies/number of progenitor cell input) x 100. Colony formation on complete media (Methocult GF M3434, Stem Cell Technology, not shown) was similar to colony formation on GM-restricted media (Figure S1B & S1C).

Differentiation on OP9 cells

Cultured cells were recovered by vigorous suspension instead of enzymatic digestion due to the potential of enzymatic destruction of epitopes which would negatively impact subsequent flow cytometric analyses. About 90% or more of cells were

recoverable by vigorous re-suspension. KSL cells typically expanded about 100-1000 fold with about 40% to 60% of the cells being B220+Gr1- (Figure S1D).

Osteoclast *In vitro* assay

To maintain healthy growth conditions, half of culture media was replaced with fresh media (αMEM + 10% serum + 10% CMG supernatant + RANKL + antibiotics) on D2 and daily afterwards. GMP cells from arthritic or control mice that are at least 6 weeks of age robustly and consistently generated osteoclasts *in vitro* and thus were used as a positive control. Note that typical protocols for generating osteoclasts *in vitro* involve priming whole bone marrow by culturing with M-CSF for about 3 days to generate what is referred to as “bone marrow macrophages”. These are then subsequently cultured with M-CSF and RANKL, to generate mature osteoclasts in about 5 more days. However to test osteoclast potential of specific cell populations as we have done in this study, the sorted cells are cultured with M-CSF and RANKL from the first day with no initial priming with M-CSF alone.

Transplantation

Recipient mice were lethally irradiated (10Gy; single dose) a day before cell transplantation. Lethally irradiated mice were administered antibiotic treated water – changed twice weekly - for 3 weeks following irradiation. Sorted cells were pelleted and resuspended in HBSS and a 200μl volume of suspension was transplanted via the tail vein. For competitive transplantation, cell suspensions from 2 competing strains were mixed at the appropriate concentrations and injected in the same 200μl volume.

Effectiveness of irradiation was verified by death of non-transplanted irradiated mice less than 2 weeks after irradiation and markedly low chimerism of recipient derived cells in transplanted mice.

We were unable to track erythroid chimerism because mature erythroid cells do not express CD45 and erythroid precursors have very low to no CD45 expression. Also even though T cell chimerism analysis was possible, these results do not reflect T lineage potential because KRNxG7 derived T cells, which express autoreactive KRN TCR, are negatively selected [1, 3]. The elimination of arthritic KSL derived T cells also makes it impossible to determine overall CD45 chimerism of arthritic versus control KSL cells although contribution to individual lineages can be assessed.

Preliminary analysis showed hardly any leukocytes in peripheral blood at very early time point (10 days or less) post transplantation. Therefore for D6 time point only bone marrow was analyzed. In fact total cell count of RBC lysed bone marrow retrieved from both tibiae and femurs of recipient mice 6 days after transplantation was about $2x10^5$ cells, about 200 fold lower than typical cellularity of unirradiated mice. At this early time point B220+ cells were also hardly existent in the bone marrow. By 16 days post transplantation, bone marrow cellularity had largely recovered with cell count of close to $3x10^7$. Mice used for 6 day- (N=4) and 16 day- (N=4) analyses were sacrificed in order to analyze bone marrow. Peripheral blood from remaining recipient mice from the same transplant cohort (N=4) was sampled non-lethally at subsequent time points (5wks, 9wks).

For transplantation into old recipients, 5 recipient mice were used. One recipient failed to engraft donor cells appreciably (<5% total donor contribution to Mac1+Gr1hi cells) and was therefore excluded from mean and standard deviation analyses presented in Figure 2D. Nevertheless, this mouse still displayed the same pattern of higher myeloid cell output from arthritic KSL

cells. Eight weeks post transplant, the PB of this recipient comprised 70% Mac1+Gr1hi cells higher than that of the other mice in the cohort (Figure S3B). Of the <5% of these Mac1+Gr1hi cells that were donor derived, 3.15% (99% of total donor derived cells) was arthritic KSL derived and only 0.026% (1% of total donor derived cells) were control KSL cell derived. On the other hand, in this same mouse, B220+ frequency at 8wks (11.4%) was lower than the other mice in the cohort, with majority of these cells coming from control KSL cells (84.5% of all, 95% of donor derived cells). Only 4.2% of the B220+ cells (5% of donor derived cells) came from arthritic mice. This and another recipient died before the 26wk analysis time point. Therefore 8wk and 16wk analyses were performed with N=4 recipients while 26wk analysis was performed with N=3 recipients.

Microarray and Bioinformatics Analysis

Total RNA was extracted and purified from FACS sorted CD45+KSL cells and RBC lysed Lin+ fraction of MACS sorted bone marrow using Arcturus Picopure RNA kit (Applied Biosystems). 3 strains were used – KRNxG7 arthritic mice, KRN non-arthritic control mice and B6xG7 non-arthritic control mice. Therefore there were 6 samples in total. For each strain, KSL cells and Lin+ cells were from 7-9 pooled mice. All mice were age matched (6-7wks). $3.7x10^5$ B6xG7 KSL, $4.7x10^5$ KSL, $7.4x10^5$ KRNxG7 KSL and $1x10^6$ Lin+ cells from each strain were used for RNA extraction. Purified RNA was linearly amplified and RNA from all 6 samples run on an Affymetrix Mouse 430 2.0 gene chip. Linear amplification and microarray were carried out at Laboratory for Clinical Genomics core facility at Washington University School of Medicine (http://pathology.wustl.edu/research/cores/lcg/index.php)

Microarray raw data was extracted and analyzed using dChip software (http://www.biostat.harvard.edu/complab/dchip/). "Upregulated genes" are at least 20% increased in arthritic KSL cells relative to *both* control KSL cell populations (from B6xG7 and KRN – both non-arthritic) examined in this study. Similarly "downregulated genes" were at least 20% reduced relative to *both* control KSL expression levels.

Clustering analysis comparing gene chips from this study to each other and other stem cell and mature cell populations from previous studies was carried out using GOurmet software [4, 5]. Briefly, average expression of genes in mature cells was determined from the three Lin+ microarray results using dChip. Lists of genes enriched in each KSL population relative to mature cells were then determined by comparing raw expression in the KSL population with the predetermined average expression of the mature cells. Fractional representation of GO terms associated with each gene list was calculated and then each cell population was then compared with previous gene expression studies at the level of the GO term representation (See Table S2).

DAVID online resource [6-8] was used for other bioinformatics analysis particularly functional annotation of differentially regulated genes.

Quantitative Real Time PCR (qRT-PCR)

For KSL sorting 3 independent arthritic mice and 3 independent control mice were used. For CD150+48-CD34-KSL cells, 3 independent pools each from arthritic and control mice were used; each pool contained cells from 2 mice. For each experiment (KSL or CD150+CD48-CD34-KSL) sorting from all mice was performed on the same day ensuring exact same sorting gates were used.

Collected cells in trizol were vortexed and stored at -80°C. Standard trizol RNA extraction according to manufacturers instruction was used extract RNA with the exception that linear acrylamide (Ambion) was used as a carrier. We estimated RNA yield to be less than 1mcg and hence all RNA extracted was used for subsequent steps including DNAse digestion and 1st Strand cDNA synthesis using Superscript III reverse transcriptase (Invitrogen). cDNA obtained was diluted in autoclaved distilled water and qRT-PCR performed with Sybr Green as done previously [3, 9]. QRT-PCR primers used in this study are listed in Table S3.

SUPPLEMENATARY TABLES

Table S1. Antibodies used in this study

Molecule	Clone	Color	Source
Kit	2B8	FITC, APC, APC-Cy7, APC-eFluor780	Biolegend, Ebioscience
Sca1	D7	PE, PerCP-Cy5.5, APC, FITC	BD, Biolegend, Ebioscience
CD3	145-2C11	FITC, PerCP-Cy5.5, APC	Biolegend, Ebioscience
CD4	GK1.5	FITC	BD, Biolegend, Ebioscience
CD4	RM4-5	PerCP-Cy5.5	Ebioscience
CD8	53-6.7	FITC, PerCP-Cy5.5	Biolegend, Ebioscience
TCRb	H57-597	PerCP-Cy5.5	Biolegend
B220	RA3-6B2	FITC, PE, PerCP-Cy5.5, APC-Cy7	Biolegend, Ebioscience
Mac1	M1/70	FITC, PE, PerCP-Cy5.5, Alexa 647	BD, Biolegend, Ebioscience
Gr1	RB6-8C5	FITC, PE, PerCP-Cy5.5, APC, APC-Cy7	BD, Biolegend, Ebioscience
Ly6G	1A8	FITC, APC	Biolegend
cFms	AFS98	PE	Biolegend, Ebioscience
Ly6C	AL-21	Biotin, FITC	BD
Ter119	Ter119	FITC, PerCP-Cy5.5	Biolegend, Ebioscience
IL-7Ra	A7R34	FITC,PerCP-Cy5.5,Biotin	Ebioscience, Biolegend
FcgR III/lib	2.4G2	PE, purified for blocking	BD
FcgR III/lib	93	Alexa 647, purified for blocking	Biolegend
CD34	RAM34	FITC, Alexa647	Ebioscience, BD
CD150	TC15-12F12.2	PE, APC, PE-Cy7	Biolegend
CD48	HM48-1	FITC, PerCP-Cy5.5	Biolegend, Ebioscience
CD11c	N418	FITC, APC	Biolegend
CD45	30-F11	FITC, PE, PerCP-Cy5.5, APC, APC-eFluor780, PE-Cy7	BD, Biolegend, Ebioscience
CD45.1	A20	FITC, PE, APC	Ebioscience, Biolegend
CD45.2	104	FITC, PE, APC	Ebioscience, Biolegend
CD71	C2	FITC	BD
CD71	R17217	APC	Ebioscience
NK1.1	PK136	PE	BD

Streptavidin	APC	BD, Ebioscience
Lineage cocktail	Biotin	Miltenyi

Table S2. References for gene chips used for dendrogram analysis.

Name	Reference
Mature Skin	10
Mature Hematopoietic Cells 1	This Study
Mature Gastric Cells 1	11
Mature Small Intestine	12
Brain	13
Mature Gastric Cells 2	14
Mature Gastric Cells 3	11
Mature Hematopoietic Cells 2	13
Mature Hematopoietic Cells 3	15
Neural Progenitors	13
Neural Progenitors	15
Gastric Progenitors	16
Mesenchymal Progenitors	17
Small Intestine Progenitors	18
Skin Progenitors	19
Hematopoietic Progenitor 4	20
Hematopoietic Progenitor 1a	This Study
Hematopoietic Progenitor 1c	This Study
Hematopoietic Progenitor 1b	This Study
Hematopoietic Progenitors 2	13
Hematopoietic Progenitors 3	15

Table S3. Quantitative RT-PCR primers used in this study

Gene	Primer Orientation	Primer Sequence	Amplicon Size (bp)	Source	
C/EBPb	f	AAGCTGAGCGACGAGTACAAGA	116		NM_009883
C/EBPb	r	GTCAGCTCCAGCACCTTGTG			
Ccr2	f	ATCCACGGCATACTATCAACATC	104	Primerbank 6753466a1	NM_009915
Ccr2	r	CAAGGCTCACCATCATCGTAG			
Chi3l3	f	AGAAGGGAGTTTCAAACCTGGT	109	Primerbank 6753416a2	NM_009892
Chi3l3	r	GTCTTGCTCATGTGTGTAAGTGA			
Ear1	f	CTGTTGGTGTGTGTGGAAATCC	119	Primerbank 6681249a3	NM_007894
Ear1	r	ATTTGTTGCCCGACTGGTGAT			
EKLF (Klf1)	f	AGACTGTCTTACCCTCCATCAG	165	Primerbank 6754454a1	NM_010635
EKLF (Klf1)	r	GGTCCTCCGATTTCAGACTCAC			
FcgrIIb	f	ATGGGAATCCTGCCGTTCCTA	117	Primerbank 6857783a3	NM_010187
FcgrIIb	r	CCCAGCAGCAAGATTTAGCAC			
FcgrIII	f	TCACTGTCCAAGATCCAGCAA	137	Primerbank 6753834a2	NM_010188
FcgrIII	r	CGGGGTTTGAAGATTTCTCCGTA			
Fog1 (Zfpm1)	f	CAGAGCCTTATCCCCTGAGAG	107	Primerbank 6677633a2	NM_009569
Fog1 (Zfpm1)	r	CGGCTTCTTCAGTTAGGACCT			
Gata1	f	ATGGAATCCAGACGAGGAAC		[9]	NM_008089
Gata1	r	CTCCCCACAATTCCCACTAC			
Ikaros	f	AGACAAGTGCCTGTCAGACAT	110	Primerbank 6677651a1	NM_009578
Ikaros	r	CCAGGTAGTTGATGGCATTGTTG			
Mpa2l	f	GTTCCAGGAAGTAACAAAGGCT	102	Primerbank 31127098a1	NM_194336
Mpa2l	r	ATCCCTAGTCTATTCCCAGTGAC			
PU.1	f	GAACAGATGCACGTCCTCGAT	107		M38252
PU.1	r	GGGGACAAGGTTTGATAAGGGAA			
Runx1	f	CTTCCTCTGCTCCGTGCTA			NM_009821
Runx1	r	CTGCCGAGTAGTTTTCATCG			
S100a8	f	AAATCACCATGCCCTCTACAAG	165	Primerbank 7305453a1	NM_013650
S100a8	r	CCCACTTTTATCACCATCGCAA			
S100a9	f	ATACTCTAGGAAGGAAGGACACC	129	Primerbank 6677837a1	NM_009114
S100a9	r	TCCATGATGTCATTTATGAGGGC			

SOCS3	f	CAAGAACCTACGCATCCAGTG	101	Primerbank 6671758a3	NM_007707
SOCS3	r	CCAGCTTGAGTACACAGTCGAA			
VWF	f	CTTCTGTACGCCTCAGCTATG	125	Primerbank 33186908a1	NM_011708
VWF	r	GCCGTTGTAATTCCCACACAAG			

SUPPLEMENTARY FIGURES

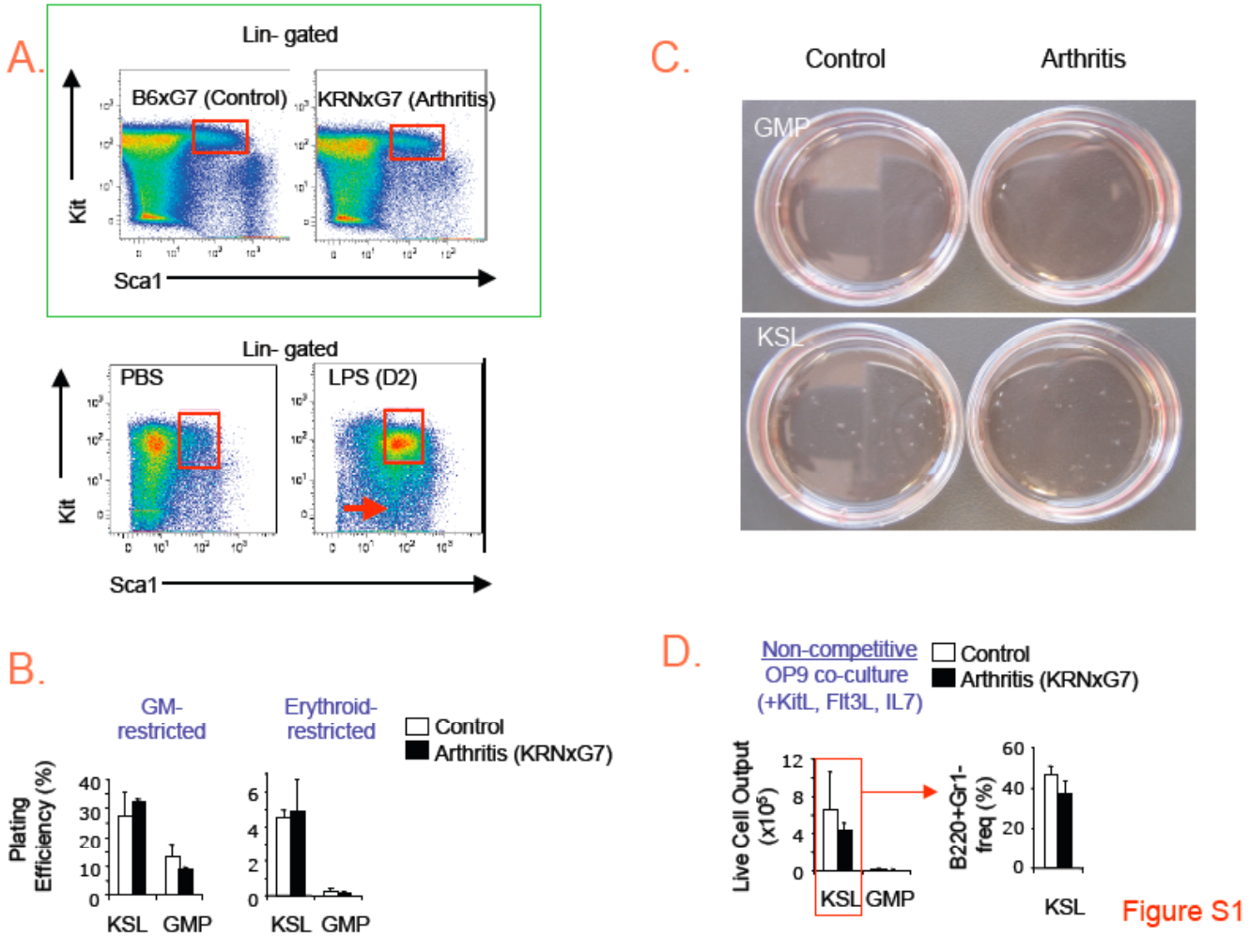


Figure S1

Figure S1. KSL phenotype identifies a similar population of bonafide uncommitted progenitors in arthritic and control mice

A. Kit-Sca plot of Lin-IL7Ra- cells from bone marrow of B6xG7 and KRNxG7 mice to indicate KSL cells used for various analyses in this study (*Top Panel*). Note that there is no "Sca-1 shift" which occurs in some models of inflammation associated with abundant IFNγ production such as following LPS injection of mice (*Bottom panel*).

B. Quantification of colonies formed by sorted BM GMP (Kit+Sca1-Lin-CD34+FcγRIII/IIb) and KSL cells in methylcellulose plates. "GM restricted" plates contain recombinant KitL, Interleukin (IL)-3 and -6 without erythropoietin (EPO). "Erythroid restricted" plates is serum free and contains EPO. (See Supplemental Experimental Proceedures for source of media).

C. "GM restricted" methylcellulose plates (day 7-8) depicting macroscopic KSL derived colonies from arthritic or control mice, visible as specks. GMP derived colonies are only visible under a microscope.

D. *Left Panel*: Cell output 6-7 days after growing 3000 sorted BM KSL cells or GMPs from arthritic and control mice on irradiated OP9 feeder cells in the presence of KitL, Flt3L and IL-7 to promote B cell differentiation.

Right Panel: B lymphocyte analysis of cells generated by KSL cells on irradiated OP9 feeder cells in the presence of KitL, Flt3L and IL-7 to promote B cell differentiation.

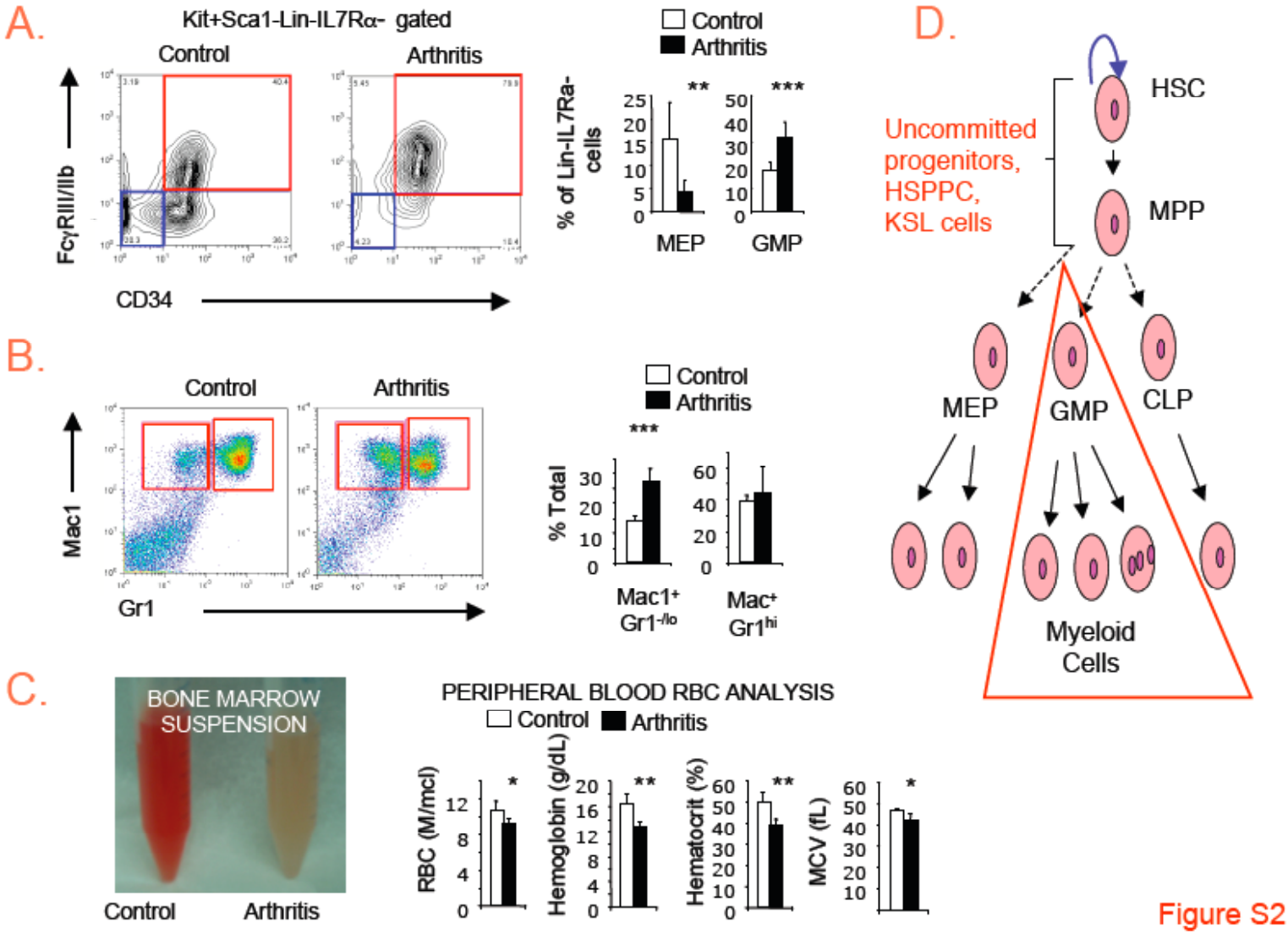

A.
Kit+Sca1-Lin-IL7Rα- gated
Control
Arthritis
FcγRIII/IIb
CD34
Control
Arthritis
% of Lin-IL7Ra- cells
**
***
MEP
GMP
B.
Control
Arthritis
Mac1
Gr1
Control
Arthritis
***
% Total
Mac1+ Gr1-/lo
Mac+ Gr1hi
C.
BONE MARROW SUSPENSION
Control
Arthritis
PERIPHERAL BLOOD RBC ANALYSIS
Control
Arthritis
RBC (M/mcl)
Hemoglobin (g/dL)
Hematocrit (%)
MCV (fL)
D.
Uncommitted progenitors, HSPPC, KSL cells
HSC
MPP
MEP
GMP
CLP
Myeloid Cells

Figure S2

Figure S2. Myeloid skewing in arthritic bone marrow

A. *Left Panel*: Representative FACS plot for analysis of BM committed progenitors. GMPs and MEPs are FcγRIII/II+CD34+ (red gate) and FcγRIII/II-CD34- (blue gate) respectively within Kit+Sca1-Lin-IL7Rα- cells.

   Right Panel: GMP and MEP frequencies normalized to total Lin-IL7Rα- myeloerythroid progenitors.

B. *Left Panel*: Representative FACS plots of Mac1 and Gr1 stained bone marrow (BM) with gated Mac1+Gr1lo and Mac1+Gr1hi populations (red gates).

   Right Panel: Mean frequency and standard deviation of BM Mac1+Gr1lo and Mac1+Gr1hi populations.

C. *Left Panel:* Marrow suspensions from tibiae and femur of control and arthritic mice. Red color is indicative of red blood cell (RBC) content in bone marrow.

   Right Panel: RBC parameters in peripheral blood analysis. Splenic extramedullary erythropoiesis (data not shown) compensates for the profound BM RBC deficit. Nevertheless, RBC counts and hemoglobin are still reduced in the peripheral blood of arthritic mice. RBC = RBC count, MCV=Mean Corpuscular Volume.

D. Pictoral description of myeloid skewing in arthritic marrow (cells in red triangle are increased relative to other cells on the same level). This paper examines if the basis of this skewing stems from uncommitted progenitors that sit at the top of this developmental hierarchy. HSC = Hematopoietic Stem Cell. MPP = Multipotential Progenitor. MEP = Megakaryocyte Erythrocyte Progenitor. GMP = Granulocyte Monocyte Progenitor. CLP = Common Lymphoid Progenitor

p<0.01*p<0.001

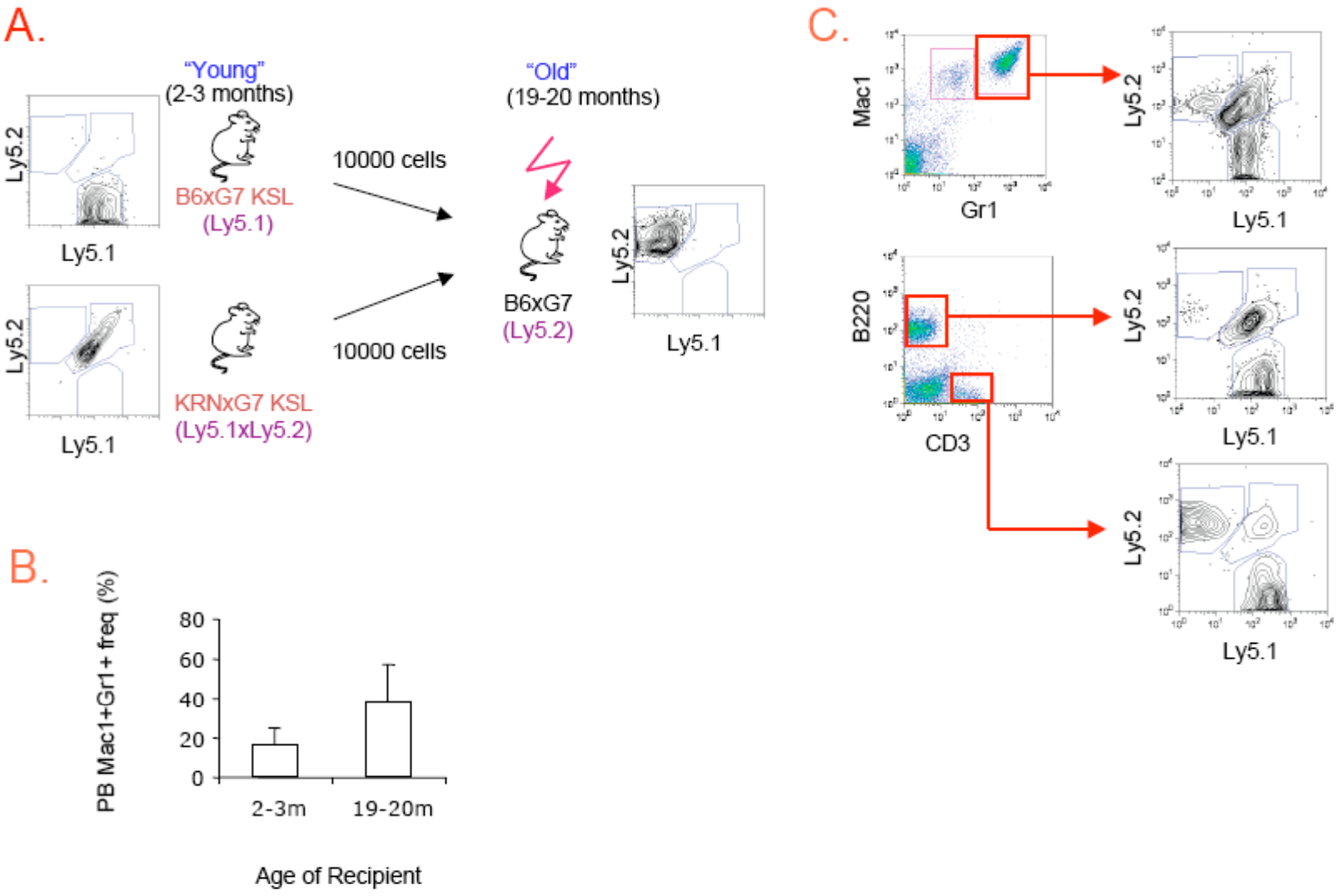

A.
"Young"
(2-3 months)
Ly5.2
Ly5.1
B6xG7 KSL
(Ly5.1)
10000 cells
Ly5.2
Ly5.1
KRNxG7 KSL
(Ly5.1xLy5.2)
10000 cells
"Old"
(19-20 months)
B6xG7
(Ly5.2)
Ly5.2
Ly5.1
B.
PB Mac1+Gr1+ freq (%)
80
60
40
20
0
2-3m
19-20m
Age of Recipient
C.
Mac1
Gr1
Ly5.2
Ly5.1
B220
CD3
Ly5.2
Ly5.1
Ly5.2
Ly5.1

Figure S3

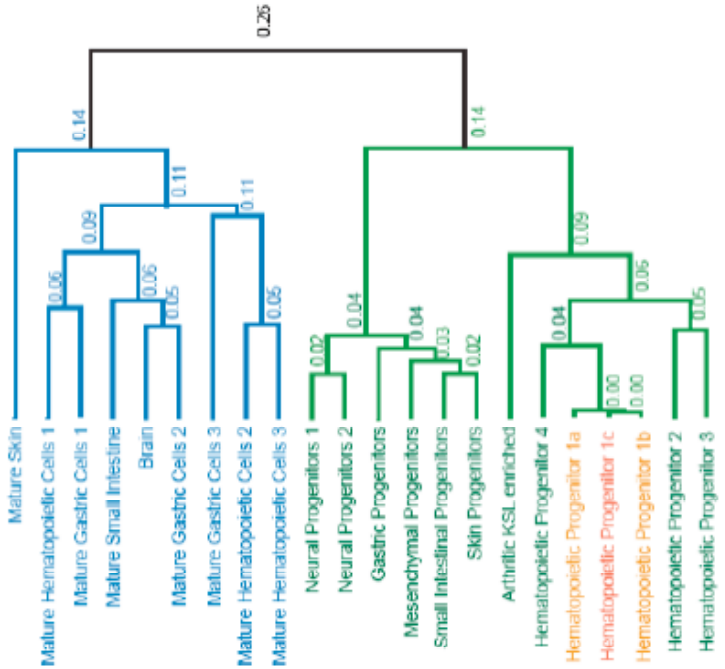
Figure S3. Mature cell analysis of transplanted mice using young and old recipients, Related to Figure 2

A. Experimental scheme for transplantation into old B6xG7 recipients. Note that CD45 alleles of B6xG7 donor, KRNxG7 donor and B6xG7 recipient mice are slightly different from what was used for the young recipient experiment.

B. Peripheral blood (PB) frequency of myeloid (Mac1+Gr1+) cells 2 months after competitive transplantation of arthritic and control KSL cells in young (2-3 month old) or old (19-20 month old) recipients.

C. Representative FACS plots showing gating scheme for analysis of myeloid cells (Mac1+Gr1hi), B cells (B220+; also CD3-) and T cells (CD3+; also B220-) in peripheral blood of recipient mice 8 weeks after transplantation. Gated cells are further analyzed for CD45.1 (Ly5.1) versus CD45.2 (Ly5.2) to determine arthritic donor derived cells (Ly5.1+) versus control donor derived cells (Ly5.1+) versus recipient derived cells (Ly5.1+Ly5.2+).

A.



B.

Category	Term	RT	Genes	Count	%	P-Value	Fold Enrichment
PIR_SUPERFAMILY	PIRSF005879:CCAAT/enhancer-binding protein	RT		3	0.7	1.8E-3	41.8
PIR_SUPERFAMILY	PIRSF005879:CCAAT/enhancer-binding	RT		3	0.7	1.8E-3	41.8
INTERPRO	CCAAT/enhancer-binding	RT		3	0.7	2.4E-3	36.7
PIR_SUPERFAMILY	PIRSF037286:Smad protein	RT		3	0.7	4.5E-3	27.9
GOTERM_BP_FAT	embryonic hemopoiesis	RT		5	1.1	3.8E-5	23.4
INTERPRO	Dwarfin	RT		3	0.7	1.1E-2	18.4
INTERPRO	SMAD domain, Dwarfin-type	RT		3	0.7	1.1E-2	18.4
INTERPRO	MAD homology, MH1	RT		3	0.7	1.1E-2	18.4
GOTERM_BP_FAT	Inactivation of MAPK activity	RT		3	0.7	1.2E-2	17.6
UP_SEQ_FEATURE	domain:MH2	RT		3	0.7	1.3E-2	16.5

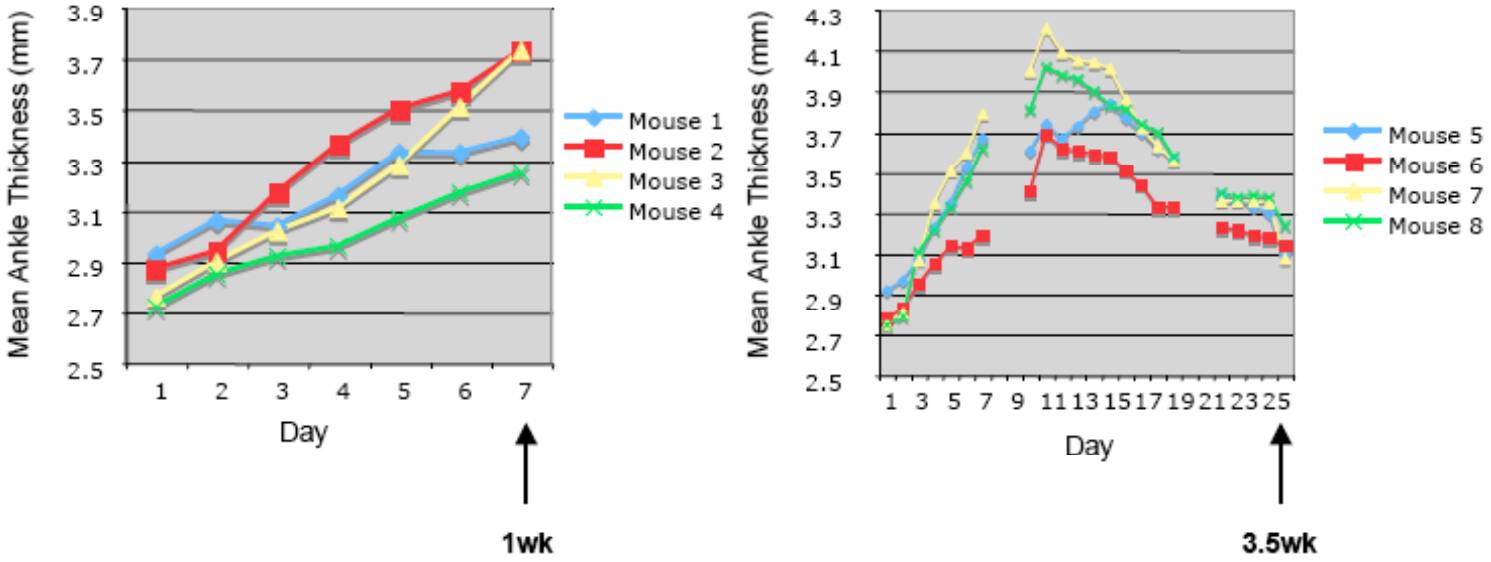
Figure S4

Figure S4. Gene Expression Analysis of Arthritic and Control KSL cells, Related to Figure 3

A. Dendrogram comparing arthritic KSL enriched expression profile ("hematopoietic progenitor 1c; red color) and control KSL enriched expression profile ("hematopoietic progenitor 1a & 1b") to each other and to profiles of stem cells (green) and mature cells (blue) from previous studies (See Table S2). Mature Lin+ expression was used as a baseline for determining KSL enriched genes. Profile of genes enriched in arthritic KSL cells relative to control KSL cells is represented by "arthritic KSL enriched". See Table S2 for references for gene chips from previous studies used in this dendrogram analysis.

B. Functional annotation terms enriched in genes upregulated in arthritic KSL cells based on DAVID Bioinformatics analysis [7]. Only terms with at least 3 genes represented in the upregulated gene list with $p<0.05$ were used in the analysis. Top 10 most enriched terms (based on "fold enrichment") are shown. Orange arrows highlight that C/EBP transcription factors are highly enriched in genes upregulated in arthritic KSL cells.

A.


Mean Ankle Thickness (mm)
3.9
3.7
3.5
3.3
3.1
2.9
2.7
2.5
1 2 3 4 5 6 7
Day
Mouse 1
Mouse 2
Mouse 3
Mouse 4
1wk
Mean Ankle Thickness (mm)
4.3
4.1
3.9
3.7
3.5
3.3
3.1
2.9
2.7
2.5
1 3 5 7 9 11 13 15 17 19 21 23 25
Day
Mouse 5
Mouse 6
Mouse 7
Mouse 8
3.5wk

B.

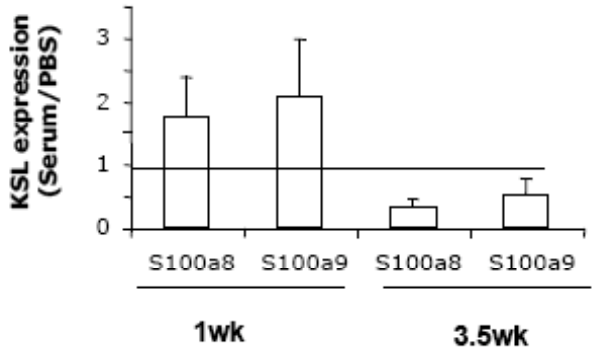

KSL expression (Serum/PBS)
3
2
1
0
S100a8
S100a9
S100a8
S100a9
1wk
3.5wk

Figure S5

Figure S5. Increased S100a8 and S100a9 expression in arthritic KSL cells is reversed during disease resolution, Related to Figure 3

A. Ankle thickness of B6 mice after injection of 250μl of arthritogenic serum. 8 mice were used 4 of which were followed for 1 week (left panel), the others for 3.5 weeks (right panel). No change in ankle thickness of control PBS injected B6 mice occurred during these periods of follow up (not shown). The cohorts used for this experiment are different from the cohort used for Figure 3D experiment.
B. S100a8 and S100a9 expression of KSL cells sorted from mice with serum transfer arthritis during active disease (1week; 1wk) and during disease resolution (3.5 weeks; 3.5wk). Expression is normalized to PBS injected controls since 1 wk and 3.5 wk mice were sacrificed on different days. The dashed line indicates equal expression in KSL cells from serum injeced and PBS injected mice.

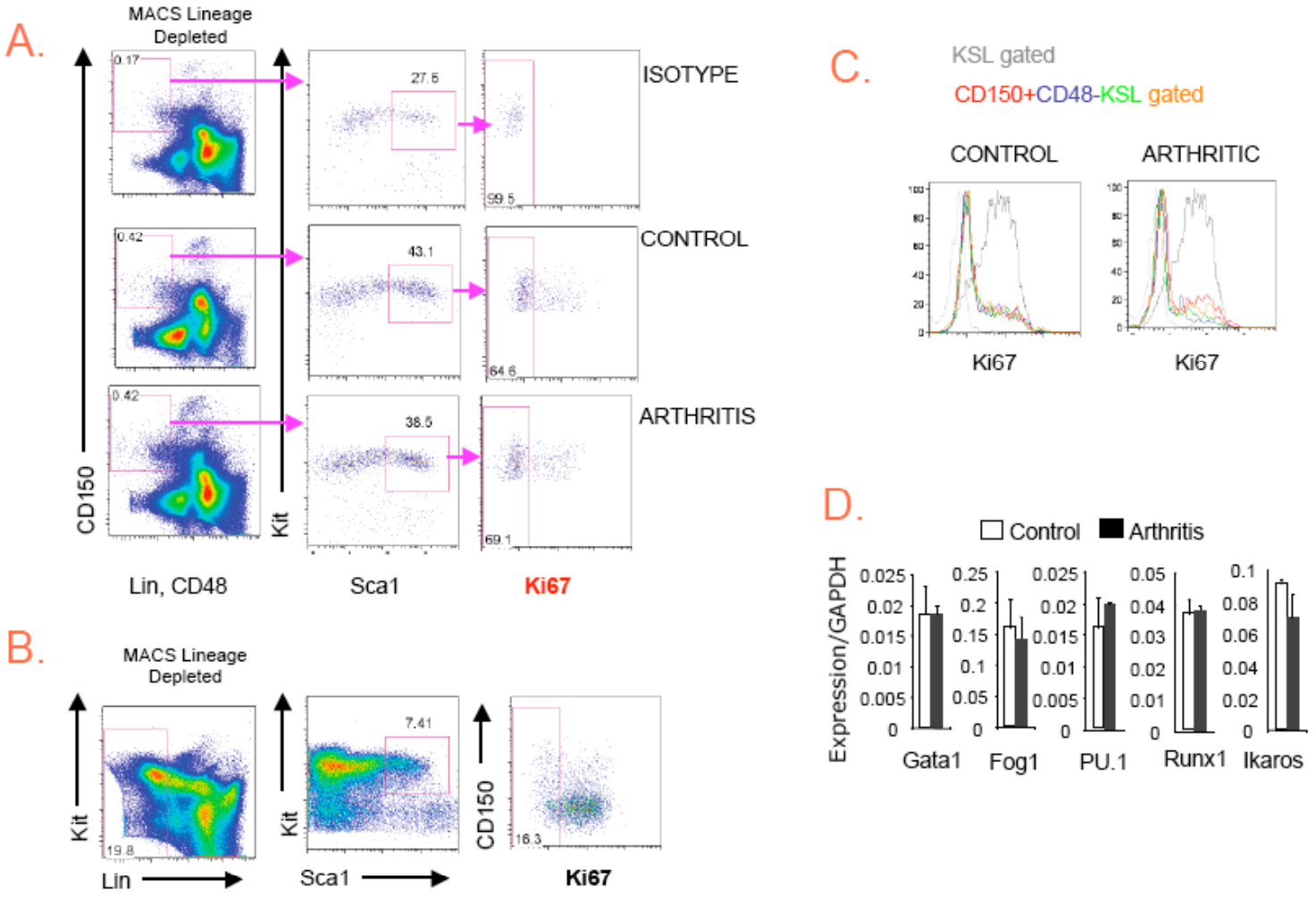

A.
MACS Lineage Depleted
ISOTYPE
CONTROL
ARTHRITIS
CD150
Kit
Lin, CD48
Sca1
Ki67
B.
MACS Lineage Depleted
Kit
Lin
Sca1
CD150
Ki67
C.
KSL gated
CD150+CD48-KSL gated
CONTROL
ARTHRITIC
Ki67
Ki67
D.
Control
Arthritis
Expression/GAPDH
Gata1
Fog1
PU.1
Runx1
Ikaros

Figure S6

Figure S6. Quiescent HSCs from arthritic and control mice express similar levels of various transcription factors, Related to Figure 4

A. Representative FACS plots depicting gating scheme for analysis of quiescent fraction of CD150+CD48-KSL IL7Rα- cells (HSCs) in bone marrow of arthritic and control mice based on intracellular Ki67 expression. An isotype control antibody (Top panel) aided in setting the Ki67 negative gate.
B. FACS plots depicting Ki67 analysis of KSL cells.
C. Most KSL cells (dark grey solid histogram) are Ki67+ (non quiescent). However most KSL CD150+CD48- cells (colored histograms) from both arthritic and control mice are Ki67- (quiescent). Light grey/hatched histogram represents isotype control.
D. Quantitative RT-PCR of transcription factors in arthritic and control HSCs (CD150+CD48-CD34-KSL IL7Rα-). Mean and standard deviation of GAPDH normalized expression is shown.

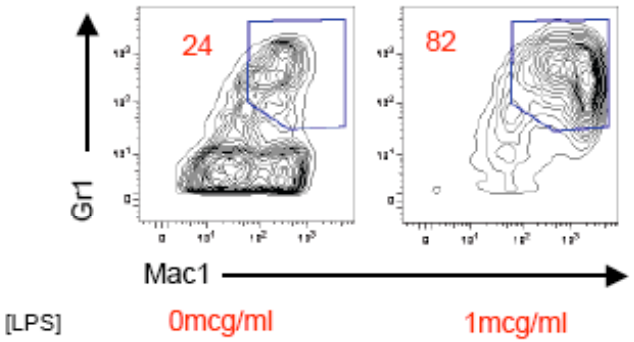


Figure S7

Figure S7. LPS promotes myeloid skewed output from arthritic KSL cells, Related to Figure 5

Naïve KSL cells from B6 mice is cultured on OP9 cells with KitL, Flt3L, IL-7 with and without LPS.

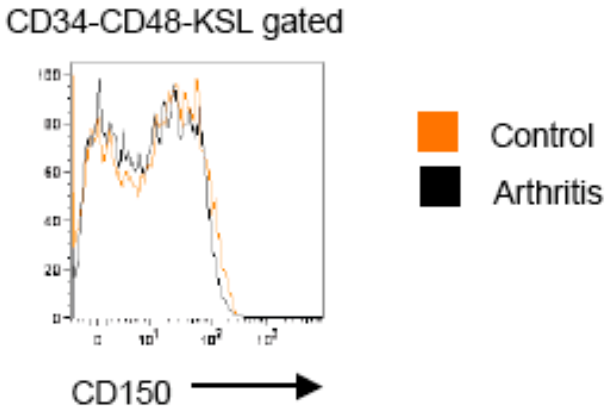


Figure S8

Figure S8. CD150 expression is similar in CD34-CD48-KSL cells from arthritic and control mice

FACS Histogram showing CD150 expression of CD34-CD48-KSL cells.

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