CD45 expression discriminates waves of embryonic megakaryocytes in the mouse

Isabel Cortegano,[1] Natalia Serrano,[2] Carolina Ruiz,[1] Mercedes Rodríguez,[1] Carmen Prado,[1] Mario Alía,[1] Andrés Hidalgo,[3] Eva Cano,[4] Belén de Andrés[1] and María-Luisa Gaspar[1]

[1]Department of Immunology, Centro Nacional de Microbiología, Instituto de Salud Carlos III (ISCIII), Majadahonda; [2]Centro de Biología Molecular Severo Ochoa, Consejo Superior de Investigaciones Científicas (CBMSO-CSIC), Madrid; [3]Area of Cell and Developmental Biology, Centro Nacional de Investigaciones Cardiovasculares, Madrid and [4]Neuroinflamation Unit, Chronic Diseases Research Program, Instituto de Salud Carlos III (ISCIII), Majadahonda, Spain

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Correspondence: *ISABEL CORTEGANO* - icortegano@isciii.es

MARÍA-LUISA GASPAR - mlgaspar@isciii.es

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Online Supplementary Methods

Embryo microsurgery and cell suspensions

Mice were sacrificed by cervical dislocation and the embryos isolated were washed with 2% fetal bovine serum (FBS) in phosphate-buffered saline (PBS). The embryo and YS were separated under a surgical stereomicroscope (Leica EZ4D), and exsanguinated in cold 2% FBS-PBS with 0.8 mg/mL heparin (Rovi Pharmaceutical Labs) to collect the peripheral blood mononuclear cells (PBMC). The FL and P-Sp/AGM were dissected out and cell suspensions were prepared by mechanical dissociation, measuring cell viability (>95%) by trypan blue dye exclusion. To analyse placenta samples, F1 mating from C57BL/6 females (expressing CD45.2 allele) with C57BL/6.CD45.1 males were used, identifying CD45-expressing cells of the embryo as CD45.1+CD45.2+. BM cell suspensions from 2-month-old mice were prepared by standard protocols.

Flow cytometry and cell purification

Cells were blocked with Fc Block (BD Biosciences) before they were incubated (4 ºC, 30 minutes) with fluorochrome-conjugated antibodies (Online Supplementary Table S2). In multicolour experiments, isotype controls for the relevant antibodies (fluorescence minus one, FMO) were included. Cell debris and dead cells were excluded by light scattering parameters and propidium iodide or 3 µM 4,6-diamino-2-phenylindole (DAPI) staining. The cell suspensions were analysed in a FACSCalibur and LSR-Fortessa-X20 with the CellQuest, DIVA (BD Biosciences) and FlowJo (Tree Star) software packages. Stained cells were purified by fluorescence-activated cell sorting (FACS) using a FACSAria (BD Biosciences) under sterile conditions, and the

purity of the cells recovered was over 98%. BM cells were purified under low pressure conditions. For cell ploidy analysis, after the staining with antibodies, cell suspensions were fixed/permeabilized in 80% ethanol and Tritón X-100 (0,1%) and the DNA was stained with DAPI. Cytospin preparations of sorted FL cells were set by centrifugation in a Cytospin-4 (65 G, 5 minutes; Shandon Southern Products) for acetylcholinesterase detection, as described.[1]

Quantitative real-time PCR

RNA was extracted and oligo(dT)-primed cDNA samples were prepared as described elsewhere.[2] Quantitative real-time polymerase chain reaction (RT-PCR) amplifications were performed in duplicate with the primers indicated in Online Supplementary Table S3, as described[3] by using a BioRad CFX cycler equipped with the CFX Manager 2.0 software, as: 1 cycle 95 °C, 10 minutes; 40 cycles of 95 °C for 10 seconds, 60 °C for 10 seconds and 72 °C for 30 seconds; a final cycle of 72 °C for 3 minutes. A melting curve was obtained and the relative amount of specific cDNA in each sample was determined by the $2^{-\Delta C_T}$ method using HPRT expression in each sample as control.

Colony-Forming Cell Assays

Cells (30-300) were plated in triplicate in 48-well plates (Nunc) in 500 µl/well of MethoCult© M3434 methylcellulose medium (Stem Cell Technologies), or in MegaCult©-C medium (Stem Cell Technologies) supplemented with 50 ng/ml rmTPO, 20 ng/ml rhIL-6, 50 ng/ml rhIL-11 (Preprotech) and 10 ng/ml rmIL-3 (Stem Cell Technologies). The plates were maintained at 37 °C in 5% CO_2 for 7-10 d and the colonies formed were counted by two independent observers under a light microscope.

Cell cultures

Purified FL cells from E11.5 embryos were plated (1-2 x 10^5 cells/cm^2) in collagen I-coated 8-well culture slides (BD Biosciences). They were cultured in 5% CO_2 for 48 h at 37 °C in DMEM-10 (Lonza) supplemented with: 10% heat-inactivated FBS, 100 U/mL penicillin-streptomycin, 2mM L-glutamine, 1% 50 µM 2-β-mercaptoethanol, 10^{-7} M dexamethasone (Sigma-Aldrich), 1 µg/mL human insulin (Novo Nordisk Pharma), 10 ng/mL murine epidermal growth factor (Peprotech), and 50 ng/mL rmTPO (Peprotech). Representative images from the cultures were captured on a Leica DMI3000B microscope equipped with a DFC420 camera (Leica).

Immunofluorescence

Immunostaining was performed on cryosections (10 µm thick) from OCT-embedded E11.5 YS and embryos, or on 8-well collagen I-coated slide culture chambers fixed in 4% paraformaldehyde. After treatment with 50 mM NH_4Cl [pH 8.0] for 10 minutes at room temperature, the slides were blocked with 2% bovine serum albumin, 0.1% Triton X-100 in PBS (room temperature, 30 minutes) and then with the Biotin Blocking System (Dako), and endogenous peroxidase activity was quenched with hydrogen peroxide. The slides were then incubated overnight at 4 °C with FITC-labelled anti-CD41 (Acris) along with biotin-labelled anti-CD45 (Becton Dickinson), or with FITC-labelled anti-CD42c (Emfret Analytics) together with biotin-labelled anti-CD41 (Acris). FITC and biotin-labelled isotype-matched antibodies (BD Biosciences) were used as controls. The signal of FITC-labelled antibodies was amplified by incubating for 45 minutes at 37 ºC with an Alexa488 conjugated anti-FITC antibody (Invitrogen, Molecular Probes). Biotin-labelled antibodies were visualized using the

Cy3-Tyramide signal-amplification system (Perkin Elmer) according to the manufacturer's indications. The preparations were mounted with Prolong Gold (Invitrogen) and analysed by confocal microscopy (Leica DMRD), processing the images with ImageJ software.

Online Supplemental Tables

Online Supplementary Table S1. Definition of the cell populations used in this study

	Surface phenotype	Antibodies used	Reference
EMK	TER119-CD41++CD42c+CD45-	TER119$^{PERCPCY5.5}$/CD41^{PE}/CD42c^{FITC}/CD45^{APCCy7}	This work
AMK	TER119-CD41++CD42c+CD45+	TER119P$^{ERCPCY5.5}$/CD41^{PE}/CD42c^{FITC}/CD45^{APCCY7}	This work
iEMK	TER119-CD41+CD45-	TER119$^{PERCPCY5.5}$/CD41^{PE}/CD42c^{FITC}/CD45^{APCCY7}	This work
iAMK	TER119-CD41+CD45+	TER119$^{PERCPCY5.5}$/CD41^{PE}/CD42c^{FITC}/CD45^{APCCY7}	This work
MKP	Lin-Sca1-cKit++CD41+CD150+FcγRII/III++	LIN/SCA1^{PECY7}/CKIT^{APC}/CD41^{FITC}/CD150$^{PERCPCY5.5}$/ FCγRII/III^{PE}	Pronk et al.[4]
PreMegE	Lin-Sca1-cKit++CD150+FcγRII/III+	LIN/SCA1^{PECY7}/CKIT^{APC}/CD41^{FITC}/CD150$^{PERCPCY5.5}$/ FCγRII/III^{PE}	Pronk et al.[4]
MEP	Lin-Sca1-cKit++CD34-FcγRII/III-	LIN/SCA1^{PECY7}/CKIT^{APC}/FCγRII/III^{FITC}/CD34^{BV421}	Akashi et al.[5]
CMP	Lin-Sca1-cKit++CD34+FcγRII/III+	LIN/SCA1^{PECY7}/CKIT^{APC}/FCγRII/III^{FITC}/ CD34^{BV421}	Akashi et al.[5]
GMP	Lin-Sca1-cKit++CD34+FcγRII/III++	LIN/SCA1^{PECY7}/CKIT^{APC}/FCγRII/III^{FITC}/CD34^{BV421}	Akashi et al.[5]
LSK	Lin-Sca1+cKit++CD135+CD34+	LIN^{PECY7}/CKIT^{APC}/SCA1^{APCCY7}/CD135^{PE}	Osawa et al.[6]
CLP	Lin-Sca1(lo)cKit+IL-7R+	LIN^{PECY7}/CKIT^{APC}/SCA1^{APCCY7}/CD127^{PE}	Kondo et al.[7]
Lin+	TER119+/CD11b+/CD90.2+/B220+/CD19+	TER119^{BIOT}/CD11b^{BIOT}/CD19^{BIOT}/B220^{BIOT}/ CD90.2^{BIOT}+STREP^{PECY7}	

Online Supplementary Table S2. Antibodies used in flow cytometry

MoAbs	Clone	Label	Company
CD9	KMC8	Biotin	BD
Mac1/CD11b	M1/70	PE/APC/Biotin	BD/BioLegend
CD31	390	PE	BD
CD34	MEC14.7	BV421	BioLegend
CD41	MWReg30	FITC/PE/APC	BD
CD42c	Xia.c3	FITC	Emfret Analytics
CD45	30F-11	FITC/APC/ APC-Cy7/PE-Cy7	BD Biolegend
CD45.1	A20	APC	Biolegend
CD45.2	104	PE-Cy7	Biolegend
CD61	2C9.G2/HMB3.1	Alexa Fluor 647	BioLegend
AA4.1/CD93	C1qRp	APC	e-Biosciences
CD105	266	PE	Miltenyi Biotec
Csf1r/CD115	AFS98	APC	BioLegend
c-Kit/CD117	2B8	FITC/APC/PE-Cy7	BD/BioLegend
CD127	A7R34	PE/Biotin	Biolegend
CD135/Flt3	Ly72/A2F10.1	PE	BD
CD150	TC15-12F12.2	PerCP-Cy5.5/BV605	BioLegend
Sca-1	E13-161.7	FITC/PE-Cy7	BD/Biolegend
TER119	Ly76	Biotin/PerCP-Cy5.5	BD/Biolegend
MPL	AMM2	Biotin	IBL-Japan
FcγRII/III	93	FITC/PE/APC-Cy7	BioLegend/ e-Biosciences
CD19	1D3	Biotin	BioLegend
CD45R/B220	22.07.11	Biotin	BioLegend
CD90.2	30-H12	Biotin	BioLegend
Streptavidin		FITC/PE/APC/ PerCP-Cy5.5/BV711	BioLegend
Isotype controls		FITC/PE/APC/ PerCP-Cy5.5/APC-Cy7/PE-Cy7/BV421	BD/Biolegend

FITC, fluorescein isothiocyanate; PE, phycoerythrin; APC, allophycocyanin, PerCP: peridinin-chlorophyll. BV: Brilliant violet. Cy: Cyanine.

Online Supplementary Table S3. Primers used for RT-qPCR

	Forward (5'-3')	Reverse (5'-3')
CD45	CTACGCAAAGCACGGCCTG	TCGAGTCTGCGTTGTCCCAC
NF-E2	GACTTGGCAAGAGATCATGT	ATATGTTGGAGGTGGCAGT
PF4	GTGTGTGAAGACCATCTCCT	CATTCTTCAGGGTGGCTAT
VWF	CCACTTGCCACAACAACATC	TTCATCCAGGATTCTCCCTG
PU1	CGGATGTGCTTCCCTTATCAAA	TGACTTTCTTCACCTCGCCTGTC
MPO	ACCTGCTGAAGAGGAAGCTG	TTGCAGTGTCCAGTGATGGT
Runx1	CCAGCAAGCTGAGGAGCGGCG	CCGACAAACCTGAGGTCGTTG
HPRT	GCCTGTATCCAACACTTCGA	TGTCATGAAGGAGATGGGAG

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Online Supplementary Figures Legends

Online Supplementary Figure S1. CD45 expression in lineage-committed progenitors from neonatal mice. Expression of CD45 by the progenitor cells in cell suspensions from the PD3 liver (PD3Li), PD3 BM (PD3BM) and adult BM. The progenitor cell populations were labelled by multicolour flow cytometry as explained in detail in Figure 3E to identify LSK ($Lin^-c\text{-}Kit^{++}Sca1^+$), CLP ($Lin^-c\text{-}Kit^+Sca1^+CD135^+$), GMPs ($Lin^-c\text{-}Kit^{++}Sca1^-CD34^+Fc\gamma RII/III^{++}$), CMPs ($Lin^-c\text{-}Kit^{++}Sca1^-CD34^{++}Fc\gamma RII/III^-$), PreMegEs ($Lin^-Sca1^-c\text{-}Kit^{++}CD150^{++}CD41^-$) and MKPs ($Lin^-Sca1^-c\text{-}Kit^{++}CD150^{++}CD41^+$). (A) The data shown in the graph are the frequencies of $CD45^+$ cells. (B) The graph shows the CD45 channel fluorescence intensity (as MFI) of $CD45^+$ cells. Data are the means ± s.e.m. (n=4 for PD3 samples and 5 for BM samples).

Online Supplementary Figure S2. CD42 and MPL expression on YS and FL cell subsets present at E10.5 and E11.5. Flow cytometry performed on preparations of E9.5-E11.5 YS and FL cell suspensions using Ter119-PerCP.Cy5.5, CD45-PE-Cy7 and CD41-PE, CD42c-FITC antibodies and MPL-Biotin/Streptavidin-APC.Cy7, in order to analyse the CD42c and MPL expression in the cell populations defined in Figure 4A after electronically excluding $Ter119^+$ cells. (A) Representative flow cytometry histograms showing the expression of MPL on R1, R2, R3 and R4 populations defined in Figure 4A at YS and FL at E11.5. The FMO isotype signal limit is shown (dotted line). (B) The bar graphs represent the frequency of cells expressing CD42c (upper graphs) and MPL (bottom graphs) in the R1-R4 populations defined in Figure 4A at E10.5 (n=5) and E11.5 (n=5) from YS (white bars) and FL (black bars). Data are the means ± s.e.m. (C) The upper contour plots are representative of the staining of YS and

FL cell preparations at E10.5 and E11.5. The boxes inside the dot plots identify cell subsets among the R2 cell population defined in Figure 4A, which are labelled R2a, R2b and R2c, as indicated in the left empty plot. Bottom bar graphs represent the frequency of $CD42c^+$ (left) and MPL^+ (right) cells on the R2a, R2b and R2c populations at E10.5 (n=2) and E11.5, n=5). Data are the means ± s.e.m. (D) The upper bar-graphs represent the percentage of cells in the R2c/$CD41^{++}CD45^+$ subset and in the R4/$CD41^{++}CD45^-$ subset (defined in Figure 4A) at E10.5 (n=2) and E11.5 (n=5). Percentages of $CD45^+$ and $CD45^-$ MKs (bottom graphs) were calculated on the basis of the CD42 expression displayed on panels C and B for the R2c/$CD41^{++}CD45^+$ and R4/$CD41^{++}CD45^-$ subsets. Data are the means ± s.e.m.

Online Supplementary Figure S3. Phenotype of $CD45^+$ and $CD45^-$ MK subsets present in the E11.5 FL. Flow cytometry performed on preparations of E11.5 FL cell suspensions using Ter119-PerCP.Cy5.5, CD45-PE-Cy7 and CD41-PE or CD41-FITC antibodies, and the antibodies indicated, in order to analyse their expression in the cell populations defined after electronically excluding $Ter119^+$ cells. (A) Strategy to define R1/$CD45^H$ ($CD45^{++}CD41^-$), R2/DP ($CD45^+CD41^+$), R3/$CD41^D$ ($CD45^-CD41^+$) and R4/$CD41^H$ ($CD45^-CD41^{++}$). The boxes inside the dot plots identify the cell subsets expressing CD45 and/or CD41. (B) Quantification of cells expressing the shown molecules (labelled with fluorochromes as indicated in Supplemental Table 2) among the CD45/CD41 subpopulations. The data are relative numbers as means ± s.e.m. (n = 3-5). These numbers are displayed as colour code in the right plot.

Online Supplementary Figure S4. BM $CD41^+CD45^+$ cells contain oligoclonal progenitors and maintain the CD45 expression after growing in culture. Adult BM

cell suspensions were stained with anti-CD45-APC, anti-CD41-PE, and anti-Ter119-PerCP.Cy5.5 and anti-CD42c-FITC, or with anti-CD45-APC, anti-CD41-PE, anti-CD42c-FITC, and biotin-labelled antibodies against Ter119, CD19, Gr1 and anti-CD3 revealed with the Streptavidin-BV711 (Lin+ cells), anti-c-Kit-PE-Cy7, anti-CD34-BV421, anti-FcγRII/III-APC-Cy7 and anti-CD150-BV605 in order to identify GMPs ($Lin^-c\text{-}Kit^{++}CD34^+Fc\gamma RII/III^{++}$), CMPs ($Lin^-c\text{-}Kit^{++}CD34^{++}Fc\gamma RII/III^-$), PreMegEs ($Lin^-c\text{-}Kit^{++}CD150^{++}CD41^{+/-}$) and MKPs ($Lin^-c\text{-}Kit^{++}CD150^{++}CD41^+$). Dead cells were excluded by light scattering parameters and propidium iodide or DAPI. (A) Left, representative contour-plot of the CD45/CD41 staining used to define $CD41^+CD45^+$ and $CD41^{++}CD45^+$ populations after electronically excluding $Ter119^+$ cells. The numbers inside are the frequency of cells in each gate, as means ± s.e.m. (n=6). The right contour-plots represent the $c\text{-}Kit^{++}$ cells in the indicated cell subpopulations. The numbers inside are the frequency of the Lin^- $c\text{-}Kit^{++}$ cells (mean ± s.e.m. n=6). (B) The graph represents the frequency of the GMPs, CMPs, PreMegEs and MKPs populations among the $Lin^-c\text{-}Kit^{++}$ cells from the $CD41^+CD45^+$ subpopulation. (C) Cells from $CD41^+CD45^+CD42c^-$ and $CD41^{++}CD45^+CD42c^+$ subpopulations isolated by flow cytometry from adult BM cell suspensions, were seeded for clonal differentiation assays in semisolid MethoCult or MegaCult medium (left and right graphs, respectively), and the colonies grown (M-CFUs and MKs-CFUs) were counted at 3, 7 and 10 days. Individual cells were detected in MegaCult cultures from $CD41^{++}CD45^+CD42c^+$ cells. The bar graphs represent the mean ± s.e.m. (n=4). (D) Cells from the $CD41^+CD45^+CD42c^-$ and the $CD41^{++}CD45^+CD42c^+$ populations indicated in panel A and the left histograms, were isolated by flow cytometry from adult BM cell suspensions, and they were cultured in short term liquid cultures (STLCs) in the presence of 50 ng/mL rmTPO in 96-well plates. After 96 hours the cells were recovered

and stained in suspension with antibodies anti-CD41-PE, anti-CD45-APC and anti-CD42c-FITC for cytometry analyses. Representative plots before the sorting procedure for each population defined in panel A are shown in the histograms for the expression of CD42c. Right, representative contour plots on $CD41^{++}CD42c^{+}$ MKs after 96 hours of culture. A representative experiment out of 5 is shown. The numbers inside are the frequency of cells in the indicated gates from the shown experiment. The mean values for $CD45^{+}$ cells are 98% ± 1% and 99% ± 1% (mean % ± s.e.m., n=5), for the $CD41^{++}CD42c^{+}$ MKs growing in STLC from $CD41^{+}CD45^{+}CD42c^{-}$ and the $CD41^{++}CD45^{+}CD42c^{+}$ cells, respectively. (E) Quantification of the DNA content in $CD41^{++}CD42c^{+}$ MKs growing in STLC of BM $CD41^{++}CD45^{+}$ cells after 96 hours (black bars, n=3) and in 48 hours STLC from the $R4/CD45^{-}CD41^{++}CD42c^{+}$ cells (white bars, n=5) from E11.5 FL cell suspensions. Data are the mean ± s.e.m.

Online Supplementary Figure S5. Phenotypic changes in surface expression markers in cultures of isolated E11.5 FL CD45-expressing cells. Flow cytometry was performed on E11.5 FL cell suspensions stained with CD45-APC-Cy7 and CD41-APC antibodies. Representative dot-plots are shown. Numbers inside the plots are the relative number of cells in the indicated gates for a representative experiment out of three performed. Fluorescence scales are logarithmic. (A) Purified $R1/CD41^{-}CD45^{++}$ cells were placed in STLC as in Figure 5, and analysed by flow cytometry at 48 hours, staining the cells with anti-c-Kit-APC, anti-CD41-PE or anti-CD42c-FITC and anti-CD11b-PE. (B) FACS-purified cells from the R2 and R3 gates indicated in the left dot-plot were analysed for CD45 expression by RT-qPCR before and after STLC performed as in Figure 5. The values for CD45 transcripts were calculated as in Figure 3D, and are displayed as means ± s.e.m. (n=3). (C) Flow cytometry was performed on E11.5 FL cell

suspensions using either anti-CD31-PE, anti-CD45-APC-Cy7, anti-CD41-APC and anti-CD42c-FITC antibodies, or only anti-CD31-PE, anti-CD41-APC and anti-CD42c-FITC antibodies. The left CD31/CD42c dot-plot defines the $CD31^{++}CD42c^{-}$ and $CD31^{++}CD42c^{+}$ cell subsets. Cells in these gates were electronically selected and their CD45 and CD41 expression was analysed in the bottom/left dot-plots, showing that these gates represent $CD45^{++}CD41^{lo}$ cells and $CD45^{-}CD41^{lo}$ cells, respectively. The middle dot-plot shows the postsort analysis of FACS-purified $CD31^{++}CD42c^{-}$ cells corresponding to the staining performed with anti-CD31-PE, anti-CD41-APC and anti-CD42c-FITC antibodies, without adding anti-CD45 and anti-CD41. These cells were placed in STLC as in Figure 5. After 48 hours the cells growing were stained with ant-CD31-PE, anti-CD45-APC-Cy7, anti-CD41-APC and anti-CD42c-FITC. The upper/right dot-plot shows the acquisition of CD42c in the culture. The bottom/right dot-plots show the CD45 and CD41 expression of electronically gated $CD31^{+}CD42c^{-}$ and $CD31^{+}CD42c^{+}$ cells. (D) RT-qPCR analyses of PF4 and MPO transcripts from cells growing in STLC after 48 hours. The cells placed in the cultures are these $CD41^{-}$ and $CD41^{d}$ indicated in the boxes in the right dot-plot. The values for each transcript were calculated as in Figure 4E, and are displayed as means ± s.e.m. (n=3).

Online Supplementary Figure S6. Definition cell subsets by flow cytometry among the CD45/CD41 cells present in the E11.5 FL. Flow cytometry was performed on preparations of E11.5 FL cell suspensions using CD41-PE, CD45-PE-Cy7, c-Kit-APC.Cy7, Ter119-PerCP.Cy5.5, CD11b-APC and CD42c-FITC antibodies. (A) Progenitor cell populations were identified in the CD45-APC-Cy7 and CD41-PE subsets defined in the left dot-plot as in Online Supplementary Figure 2. c-Kit-APC and Lin-PE-Cy7 expression is shown in the right dot-plot. The data shown are from a

representative E11.5 FL preparation. (B) Cells in the gates depicted in the left dot-plot in panel A were electronically selected and the c-Kit/Ter119 expression was analysed (upper dot-plots) among the R1, R2, R3 and R4 populations. c-Kit$^{+/-}$Terr119^{-} cells were electronically gated for each of these populations, and analysed subsequently for CD11b and CD42c expression in the bottom dot-plots. The horizontal dotted lines in the upper dot-plots represent the isotype control limit of the c-Kit antibody. The numbers inside the bottom plots are percentages of the gated CD11b^{+} and CD42c^{+} populations. A representative experiment of three performed is shown. Fluorescence scales are logarithmic.

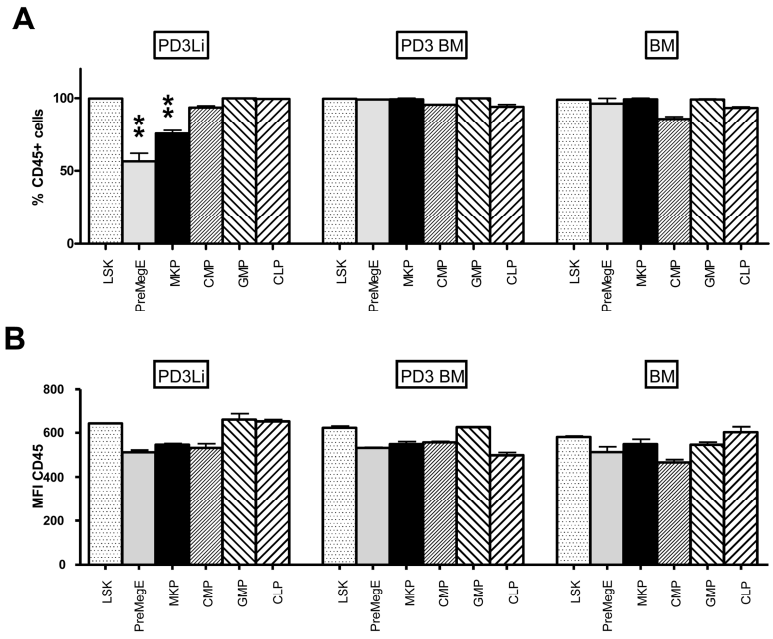
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Fig. S1

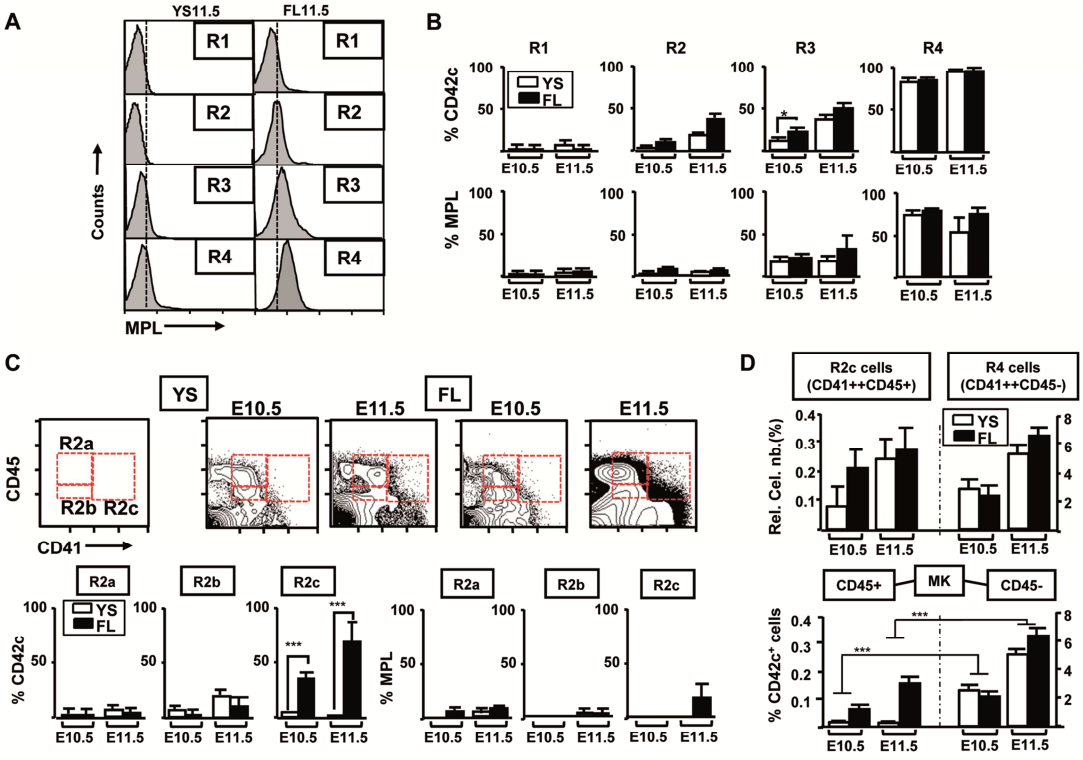
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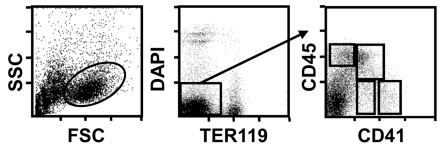
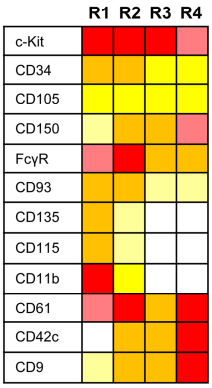
Fig. S2

A



B

	R1/CD45^{H}	R2/DP	R3/CD41^{D}	R4/CD41^{H}
c-Kit	96,4 ± 0,5	96,2 ± 3,3	95,2 ± 2,7	77,2 ± 4,0
CD34	46,8 ± 17	25,9 ± 5,9	11,8 ± 3,8	24,6 ± 3,4
CD105	15,2 ± 0,7	14,5 ± 1,7	15,9 ± 0,8	10,7 ± 3,1
CD150	4,3 ± 1,3	41 ± 14	33,6 ± 7,2	67,7 ± 12,8
FcγR	77.7 ± 2.2	83.9 ± 4.6	33 ± 3.5	47.1 ± 7.6
CD93	38,4 ± 1,6	28,9 ± 3,6	4,2 ± 0,1	4,6 ± 0,8
CD135	37,9 ± 3,2	1,1 ± 0,5	0,9 ± 0,1	0,25 ± 0,1
CD115	35,1 ± 4,8	1,7 ± 1,2	0,3 ± 0,2	0,53 ± 0,4
CD11b	84,4 ± 5,7	10,6 ± 3,2	0,6 ± 0,4	0,67 ± 0,2
CD61	60 ± 3,1	84,8 ± 1,9	43,7 ± 1,9	97,9 ± 0,1
CD42c	0,05 ± 0,09	32,2 ± 8,1	37,5 ± 5,6	94 ± 1,6
CD9	6.2 ± 1.9	40.1 ± 1.1	45.7 ± 4.9	97.4 ± 0.5



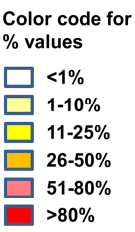


Fig. S3

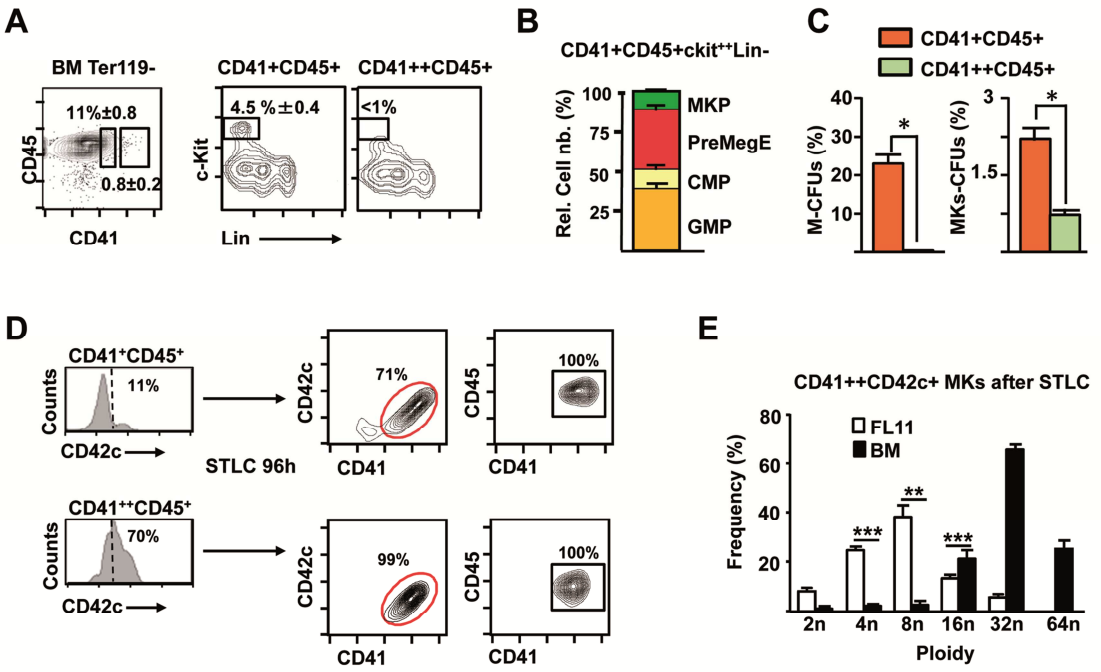

A
BM Ter119-
CD41+CD45+
CD41++CD45+
11%±0.8
0.8±0.2
CD45
CD41
4.5 %±0.4
<1%
c-Kit
Lin
B
CD41+CD45+ckit++Lin-
Rel. Cell nb. (%)
100
75
50
25
MKP
PreMegE
CMP
GMP
C
CD41+CD45+
CD41++CD45+
M-CFUs (%)
40
30
20
10
MKs-CFUs (%)
3
1.5
*
*
D
CD41+CD45+
Counts
11%
CD42c
STLC 96h
71%
CD42c
CD41
100%
CD45
CD41
CD41++CD45+
70%
99%
100%
E
CD41++CD42c+ MKs after STLC
FL11
BM
Frequency (%)
80
60
40
20
***
**
***
2n
4n
8n
16n
32n
64n
Ploidy

Fig. S4

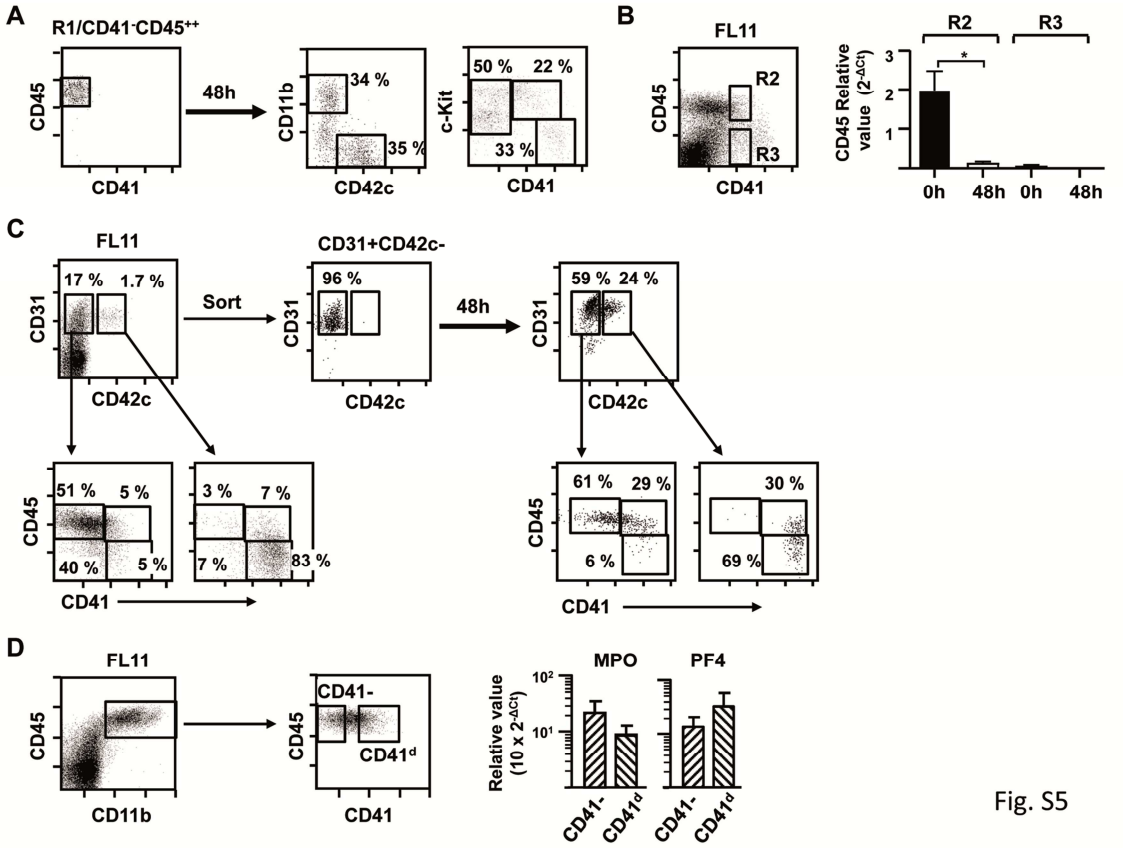

A
R1/CD41-CD45++
CD45
CD41
48h
CD11b
34 %
35 %
CD42c
c-Kit
50 %
22 %
33 %
CD41
B
FL11
CD45
R2
R3
CD41
CD45 Relative value (2-ΔCt)
R2
R3
*
0h
48h
0h
48h
C
FL11
CD31
17 %
1.7 %
CD42c
Sort
CD31+CD42c-
96 %
48h
59 %
24 %
51 %
5 %
40 %
5 %
3 %
7 %
7 %
83 %
61 %
29 %
6 %
30 %
69 %
CD41
D
FL11
CD45
CD11b
CD41-
CD41d
Relative value (10 x 2-ΔCt)
MPO
PF4
CD41-
CD41d
CD41-
CD41d

Fig. S5

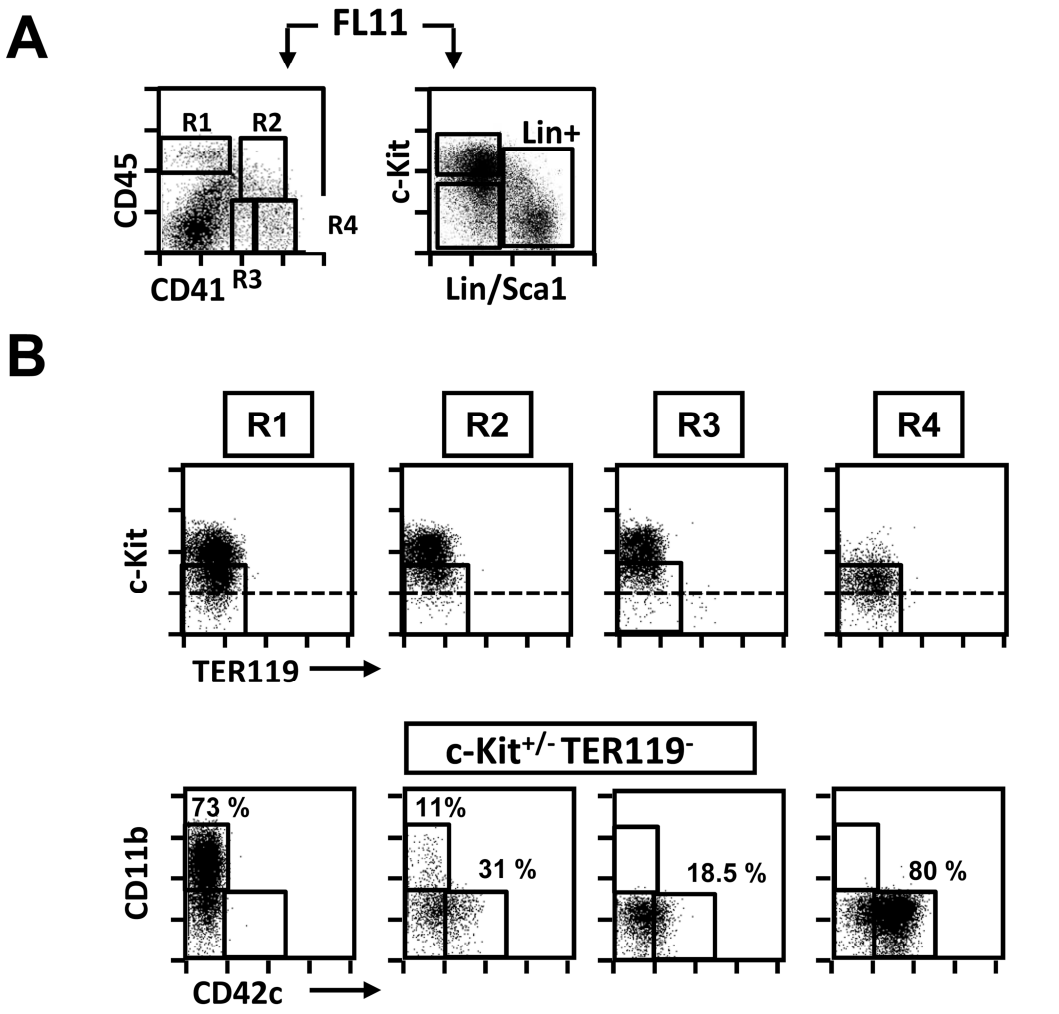

A
FL11
R1
R2
R4
R3
CD45
CD41
c-Kit
Lin+
Lin/Sca1
B
R1
R2
R3
R4
c-Kit
TER119
c-Kit+/- TER119-
CD11b
CD42c
73 %
11%
31 %
18.5 %
80 %

Fig. S6