SUPPLEMENTARY APPENDIX

Clonal tracking of erythropoiesis in rhesus macaques

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Supplementary Materials

Supplementary methods

Barcoded library diversity

Diversity of each barcoded lentiviral library preparation was validated using Monte Carlo simulations of retrieved barcodes to determine the number of $CD34^+$ target cells able to be transduced with each library preparation resulting in a greater than 95% probability that more than 95% of barcodes represent transduction of single engrafting cells(2, 5, 6).

Barcode retrieval

DNA was extracted from all cell collections with the DNeasy Blood & Tissue kit (Qiagen) and quantified by Qubit (Invitrogen). RNA from nucleated cells was extracted using RNAzol®RT (Molecular Research Center)(8). RNA from enucleated mature RBC and reticulocytes was extracted using PAXgene Blood RNA Tubes and PAXgene Blood RNA Kits (PreAnalytiX). RNA was reverse transcribed using the SuperScript™ IV First-Strand Synthesis System (Thermo Fisher). 200 ng (ZH33, ZG66, ZJ31, ZK22, JD76 and ZL40) or 500 ng (ZH19) DNA, or cDNA reverse transcribed from 12ng RNA underwent PCR with primers (Table S2) bracketing the barcode and multiplex sequencing as described(2, 6). Equal amounts of gel-purified barcode PCR product from individual samples were pooled together for multiplex sequencing (Illumina HiSeq 3000).

Sequencing data analysis

Data analysis, Pearson correlations, Euclidean distances, *P* values, plot generation and statistical analyses were performed using R (Foundation for Statistical Computing) and Prism (GraphPad Software). Python code and associated R functions used for analyses are available on Github (www.github.com/dunbarlabNIH/).

References

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Table S1: Antibodies used for flow cytometric sorting of blood and bone marrow cell populations.

Antigen	Conjugation	Vendor	Catalog number	Clone
CD3	APC-Cy7	BD Pharmingen	557757	SP34-2
CD3	BV786	BD Pharmingen	563918	SP34-2
CD14	Pacific blue	Invitrogen	MHCD1428	TUK4
CD20	BV650	BD Pharmingen	563780	2H7
CD20	APC Cy7	BD Pharmingen	335794	L27
CD34	PE	BD Pharmingen	550761	563
CD34	Purified (for $CD34^+$ selection)	N/A	N/A	12.8
CD45	BV510	BD Horizon	563830	D058-1283
CD71	PE	GeneTex	GTX43030	DF1513

Table S2: Sequences of primers used for barcodes retrieval.

Primer Names	5’----------3’ sequences	Index
Universal reverse primer	CAAGCAGAAGACGGCATACGAGATCGTGATACGGCATACGAGCTCTTCCGATCT	
Barcode New Forward i501	AATGATACGGCGACCACCGAGATCTACACTCGCCTTAACACTCTTTCCCTACACGACGCTCTTCCGATCT	TCGCCTTA
Barcode New Forward i502	AATGATACGGCGACCACCGAGATCTACACCTAGTACGACACTCTTTCCCTACACGACGCTCTTCCGATCT	CTAGTACG
Barcode New Forward i503	AATGATACGGCGACCACCGAGATCTACACTTCTGCCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	TTCTGCCT
Barcode New Forward i504	AATGATACGGCGACCACCGAGATCTACACGCTCAGGAACACTCTTTCCCTACACGACGCTCTTCCGATCT	GCTCAGGA
Barcode New Forward i505	AATGATACGGCGACCACCGAGATCTACACAGGAGTCCACACTCTTTCCCTACACGACGCTCTTCCGATCT	AGGAGTCC
Barcode New Forward i506	AATGATACGGCGACCACCGAGATCTACACCATGCCTAACACTCTTTCCCTACACGACGCTCTTCCGATCT	CATGCCTA
Barcode New Forward i507	AATGATACGGCGACCACCGAGATCTACACGTAGAGAGACACTCTTTCCCTACACGACGCTCTTCCGATCT	GTAGAGAG
Barcode New Forward i510	AATGATACGGCGACCACCGAGATCTACACCAGCCTCGACACTCTTTCCCTACACGACGCTCTTCCGATCT	CAGCCTCG
Barcode New Forward i511	AATGATACGGCGACCACCGAGATCTACACTGCCTCTTACACTCTTTCCCTACACGACGCTCTTCCGATCT	TGCCTCTT
Barcode New Forward i512	AATGATACGGCGACCACCGAGATCTACACTCCTCTACACACTCTTTCCCTACACGACGCTCTTCCGATCT	TCCTCTAC
Barcode New Forward i514	AATGATACGGCGACCACCGAGATCTACACTCATGAGCACACTCTTTCCCTACACGACGCTCTTCCGATCT	TCATGAGC
Barcode New Forward i515	AATGATACGGCGACCACCGAGATCTACACCCTGAGATACACTCTTTCCCTACACGACGCTCTTCCGATCT	CCTGAGAT
Barcode New Forward i516	AATGATACGGCGACCACCGAGATCTACACTAGCGAGTACACTCTTTCCCTACACGACGCTCTTCCGATCT	TAGCGAGT
Barcode New Forward i518	AATGATACGGCGACCACCGAGATCTACACGTAGCTCCACACTCTTTCCCTACACGACGCTCTTCCGATCT	GTAGCTCC
Barcode New Forward i519	AATGATACGGCGACCACCGAGATCTACACTACTACGCACACTCTTTCCCTACACGACGCTCTTCCGATCT	TACTACGC
Barcode New Forward i520	AATGATACGGCGACCACCGAGATCTACACAGGCTCCGACACTCTTTCCCTACACGACGCTCTTCCGATCT	AGGCTCCG
Barcode New Forward i521	AATGATACGGCGACCACCGAGATCTACACGCAGCGTAACACTCTTTCCCTACACGACGCTCTTCCGATCT	GCAGCGTA
Barcode New Forward i522	AATGATACGGCGACCACCGAGATCTACACCTGCGCATACACTCTTTCCCTACACGACGCTCTTCCGATCT	CTGCGCAT
Barcode New Forward i523	AATGATACGGCGACCACCGAGATCTACACGAGCGCTAACACTCTTTCCCTACACGACGCTCTTCCGATCT	GAGCGCTA
Barcode New Forward i524	AATGATACGGCGACCACCGAGATCTACACCGCTCAGTACACTCTTTCCCTACACGACGCTCTTCCGATCT	CGCTCAGT
Barcode New Forward i526	AATGATACGGCGACCACCGAGATCTACACGTCTTAGGACACTCTTTCCCTACACGACGCTCTTCCGATCT	GTCTTAGG
Barcode New Forward i527	AATGATACGGCGACCACCGAGATCTACACACTGATCGACACTCTTTCCCTACACGACGCTCTTCCGATCT	ACTGATCG
Barcode New Forward i528	AATGATACGGCGACCACCGAGATCTACACTAGCTGCAACACTCTTTCCCTACACGACGCTCTTCCGATCT	TAGCTGCA
Barcode New Forward i529	AATGATACGGCGACCACCGAGATCTACACGACGTCGAACACTCTTTCCCTACACGACGCTCTTCCGATCT	GACGTCGA

Supplementary Figure Legends

Figure S1: Purification strategies for HSPCs and specific hematopoietic lineages

(A) Representative purity check by flow cytometric analysis of BM $CD34^+$ HSPCs from RM ZK22 15.5 months post-transplantation selected by using MACS beads.

(B) Schematic of flow cytometric gating strategy used for sorting lineage-specific cells from the BM samples (upper panel), and the FACS plots (lower panel) of ZL40 BM sample at 15m.

(C) Schematic of flow cytometric gating strategy used for sorting lineage-specific cells from the PB sample (upper panel), and the FACS plots (lower panel) of PB sample from ZL40 at 15m.

Figure S2: Lineage bias of contributing clones

Bar plots displaying the bias and relative size of each barcode. Each barcode is represented as a grey box with a black outline so that very small clones appear only as a black line. The categories in which clones fall are determined by comparing the percent contribution of the given lineage on the left to the maximum percent contribution of all other lineages (NRBC, Gr, Mono, $CD34^+$, T, B without the given lineage). The positive sign (+) indicates bias towards the given lineage (i.e. present more in the NRBC lineage) and the negative sign (-) indicates bias away from the given lineage (i.e. present more in other lineages).

Figure S3: GFP expression in individual blood cell lineages

(A)The FACS plots of CD45 and GFP expression of the PB RBC and granulocytes (Gr) in both MSCV- and EF1a-drivened barcode lentivirus transduced HSPC RM.

(B) Bar plot summarized the GFP% in RBCs and Gr in individual rhesus macaques. Different colors indicate different cell types.

Figure S4: Nuclear red blood cell (NRBC) percentage in bone marrow samples

The FACS plots show NRBCs' sorting strategy of CD45-CD71+ in different RM's bone marrow samples.

Figure S1

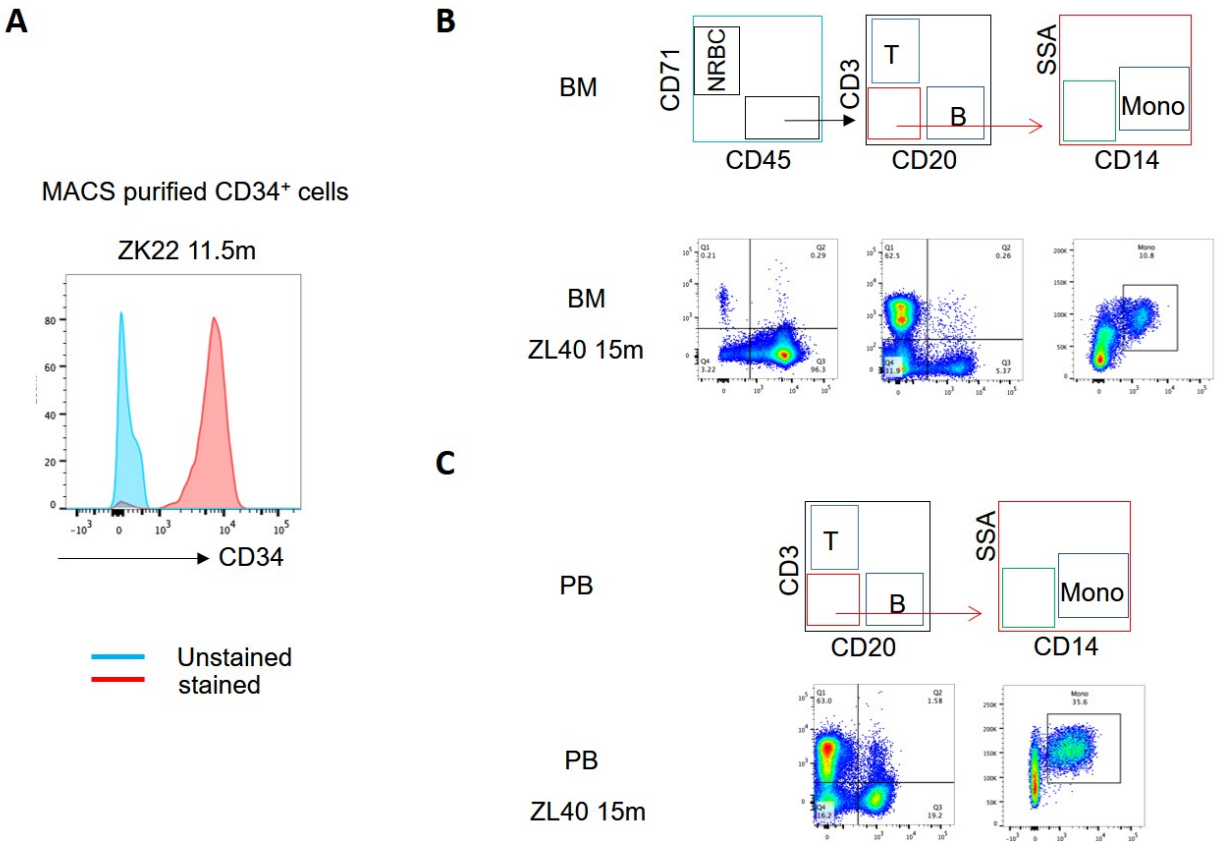

A
MACS purified CD34+ cells
ZK22 11.5m
CD34
Unstained
stained
B
BM
CD71
NRBC
CD45
CD3
T
B
CD20
SSA
Mono
CD14
BM
ZL40 15m
C
PB
CD3
T
B
CD20
SSA
Mono
CD14
PB
ZL40 15m

Figure S2

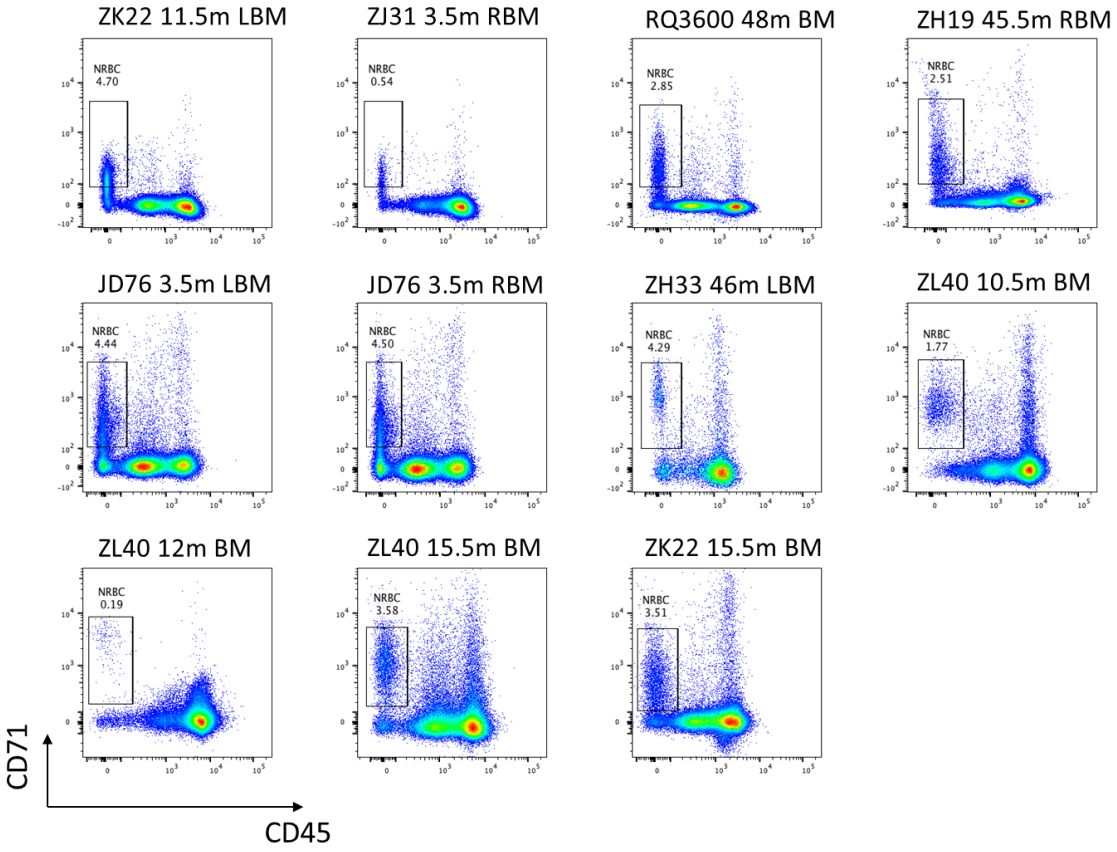

ZK22 11.5m LBM
NRBC
4.70
ZJ31 3.5m RBM
NRBC
0.54
RQ3600 48m BM
NRBC
2.85
ZH19 45.5m RBM
NRBC
2.51
JD76 3.5m LBM
NRBC
4.44
JD76 3.5m RBM
NRBC
4.50
ZH33 46m LBM
NRBC
4.29
ZL40 10.5m BM
NRBC
1.77
ZL40 12m BM
NRBC
0.19
ZL40 15.5m BM
NRBC
3.58
ZK22 15.5m BM
NRBC
3.51
CD71
CD45

Figure S3

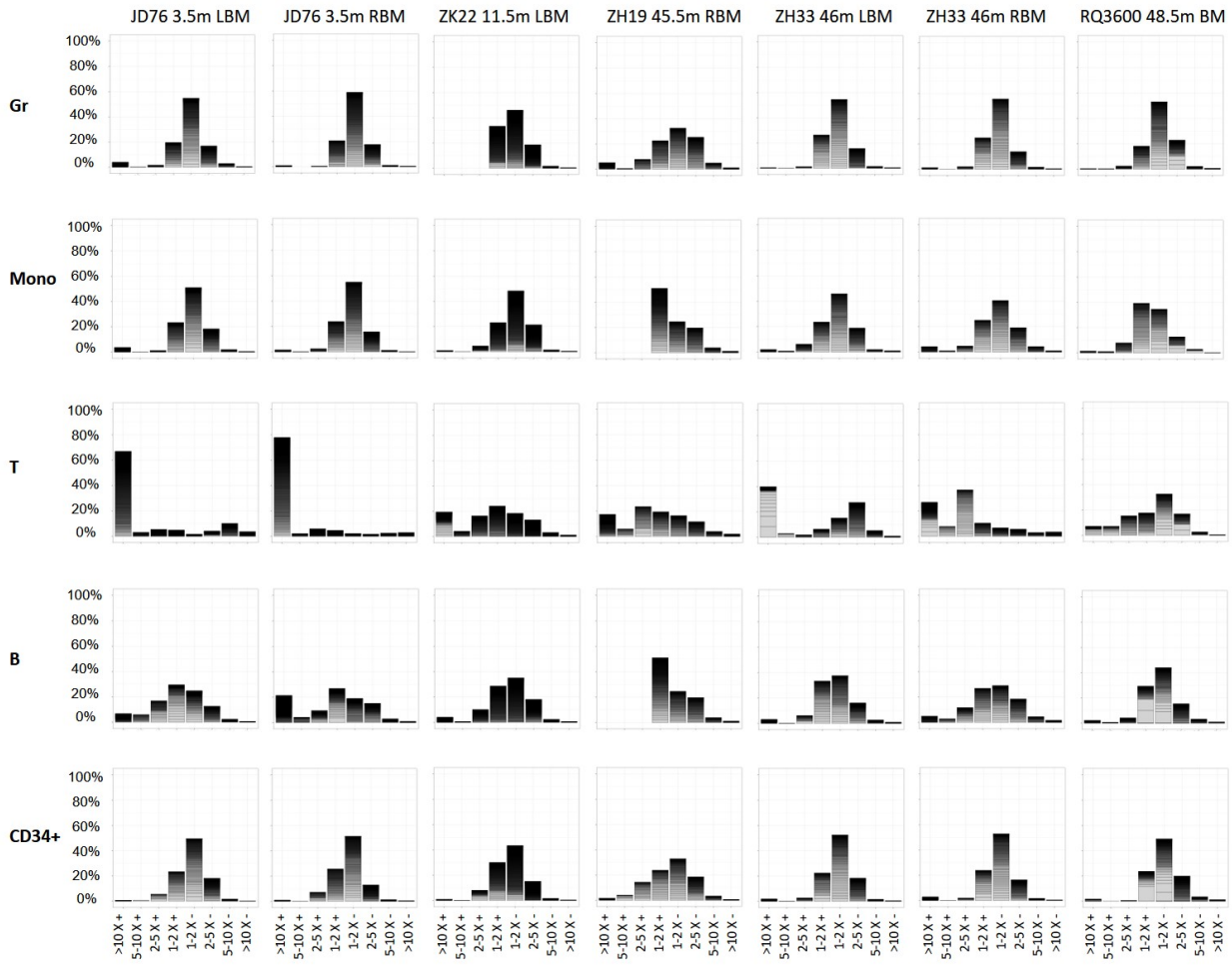
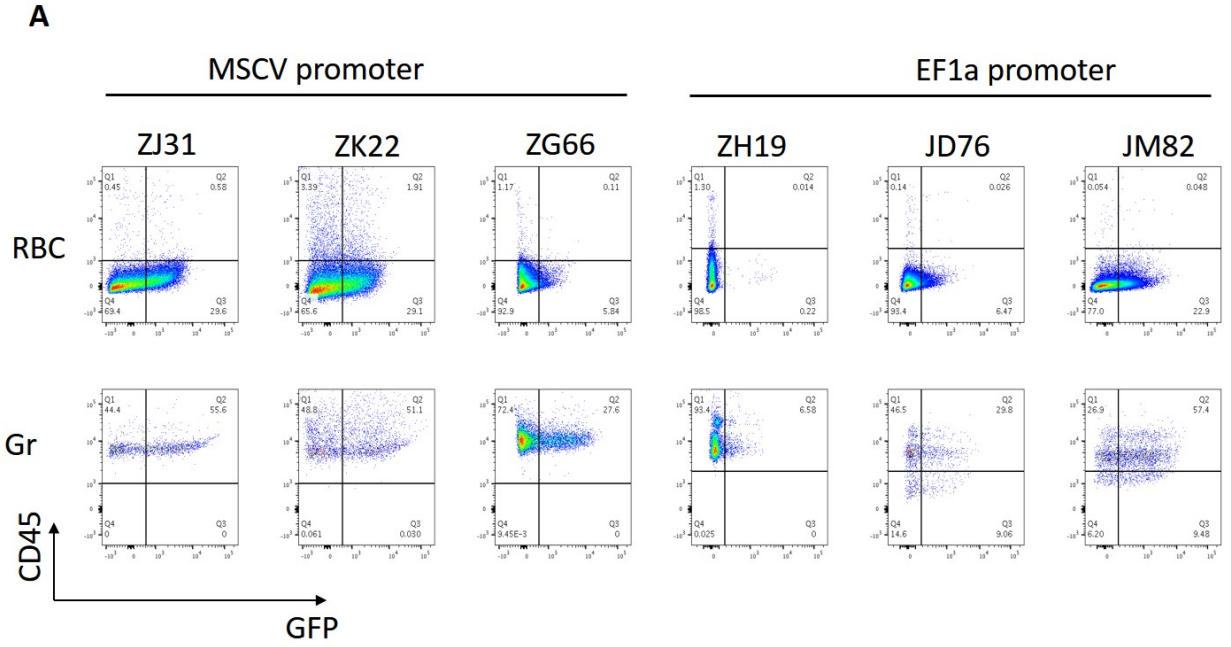
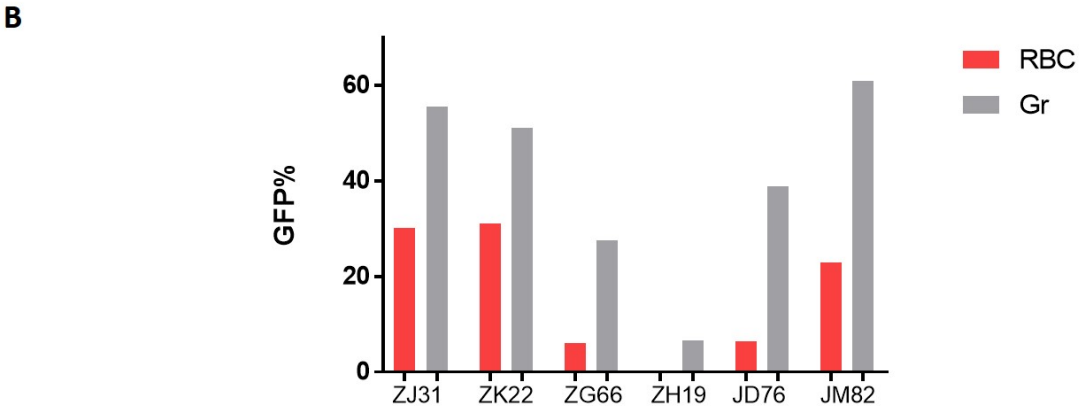

JD76 3.5m LBM
JD76 3.5m RBM
ZK22 11.5m LBM
ZH19 45.5m RBM
ZH33 46m LBM
ZH33 46m RBM
RQ3600 48.5m BM
Gr
Mono
T
B
CD34+
100%
80%
60%
40%
20%
0%
>10 X +
5-10 X +
2-5 X +
1-2 X +
1-2 X -
2-5 X -
5-10 X -
>10 X -

Figure S4


A
MSCV promoter
EF1a promoter
ZJ31
ZK22
ZG66
ZH19
JD76
JM82
RBC
Gr
CD45
GFP


B
GFP%
60
40
20
0
ZJ31
ZK22
ZG66
ZH19
JD76
JM82
RBC
Gr