**Supplemental Methods**

**Production of lentiviral barcoded library**: The lentiviral barcode libraries were prepared as previously described.[1,2] Briefly, a 6 bp library identifier followed by 27 or 35 bp semi-random DNA sequences were synthesized and cloned into the expression cassette following the GFP marker gene in the HIV-derived, replication-defective pCDH vector from System Biosciences (Mountain View, CA). Highly diverse barcoded lentiviral libraries were transfected into 293T cells together with χHIV,[3] HIV Rev/Tat, and VSV-G envelope expression plasmids, and viral supernatants were collected post-transfection. Each batch of vector was assayed for titer, potency in transduction of HSPC, and Monte Carlo simulations were performed to assess barcode diversity, determining the number of target cells able to be transduced with each lentiviral library, ensuring with greater than 95% probability that more than 95% of barcodes represent single transduced cell. Individual vector preparations with distinct library IDs were utilized for the two aged animals transplanted in this study.

**HSPC transduction and autologous transplantation**: All procedures were approved by the NHLBI Animal Care and Use Committee. Mobilization, HSPC collection, and immunoselection were performed as previously described.[2,4] Health of both the young and aged animals was monitored before and after transplantation was monitored via at least quarterly physical exams and routine monitoring of blood counts and chemistries. HSPC were cultured in X-VIVO™ 10 (Lonza, Walkersville, MD) supplemented with 1% HSA (Baxter, Deerfield, IL) and cytokines (SCF 100ng/mL, FLT3L 100ng/mL and TPO 100ng/mL, PeproTech, Rocky Hill, NJ). HSPC were transduced once at a multiplicity of infection of 25 in the presence of 4 mg/ml protamine sulfate (Sigma, St. Louis, MO). Transduced HSPC were reinfused into each autologous macaque following

total body irradiation (500 cGy/day for 2 days). Information regarding barcode transduction and HSPC dose for young and aged animals are summarized in Figure 1B.

**Hematopoietic cell processing**: Rhesus macaque PB was processed via centrifugation over Lymphocyte Separation Medium (MP Biomedicals, Santa Ana, CA). Cells were isolated from inguinal or axillary lymph nodes (LN) by gentle mechanical disruption and passed through a 100 µm filter to remove tissue fragments.[5] Cells were stained with antibodies as listed in Table S1. Flow cytometric analysis and/or sorting were performed on a FACSARIA-II flow sorter (BD Bioscience, Heidelberg, Germany), and data were analyzed using FlowJo software (Tree-star Inc., Ashland, OR). Genomic DNA from hematopoietic cells was extracted using DNeasy Blood & Tissue kits (Qiagen, Germantown, MD).

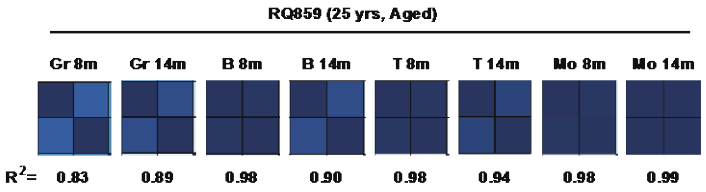
## Supplemental References


1. Lu R, Neff NF, Quake SR, Weissman IL. Tracking single hematopoietic stem cells in vivo using high-throughput sequencing in conjunction with viral genetic barcoding. *Nature biotechnology*. 2011;29(10):928-933.
2. Wu C, Li B, Lu R, et al. Clonal tracking of rhesus macaque hematopoiesis highlights a distinct lineage origin for natural killer cells. *Cell Stem Cell*. 2014;14(4):486-499.
3. Uchida N, Washington KN, Hayakawa J, et al. Development of a human immunodeficiency virus type 1-based lentiviral vector that allows efficient transduction of both human and rhesus blood cells. *J Virol*. 2009;83(19):9854-9862.
4. Donahue RE, Kuramoto K, Dunbar CE. Large animal models for stem and progenitor cell analysis. *Curr Protoc Immunol*. 2005;Chapter 22:Unit 22A 21.
5. Lore K, Seggewiss R, Guenaga FJ, et al. In vitro culture during retroviral transduction improves thymic repopulation and output after total body irradiation and autologous peripheral blood progenitor cell transplantation in rhesus macaques. *Stem Cells*. 2006;24(6):1539-1548.

**Table S1.**

| name | fluorochrome | clone | Company | cat # |
|---|---|---|---|---|
| CD3 | APC-cy7 | SP34-2 | BD Biosciences | 557757 |
| CD20 | PE-cy5 | 2H7 | BD Biosciences | 555624 |
| CD56 | PE | B159 | BD Biosciences | 555516 |
| CD14 | Pacific Blue | TüK4 | Invitrogen | MHCD1428 |
| CD16 | APC | 3G8 | Biolegend | 302012 |
| CD4 | BV650 | SK3 | BD Biosciences | 563875 |
| CD8 | PE-cy7 | SIDI8BEE | eBioscience | 25-5273-42 |
| CD33 | PE | AC104.3E3 | Miltenyi Biotec | 130-091-732 |

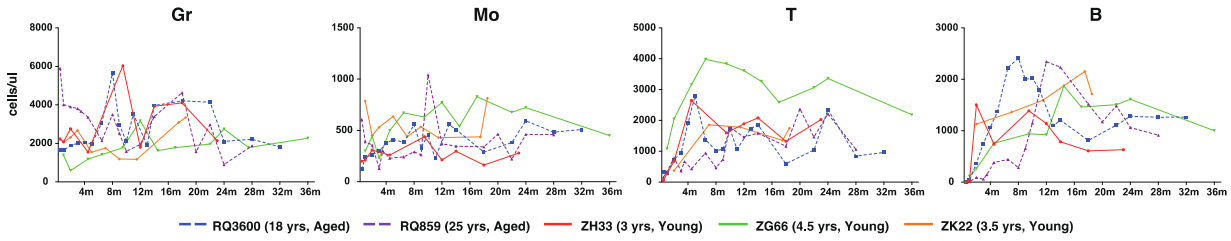
**Supplementary Figure 1**




**Supplementary Figure 1. Reproducibility of barcode retrieval from replicate samples**

Sorted Gr, B, T, and Mo duplicate samples obtained at 8 and 14 months in RQ859 were run through two independent PCR followed by Illumina sequencing reactions, and analysis are shown. The correlation between all barcode contributions in the replicates are shown as $r^2$ values.

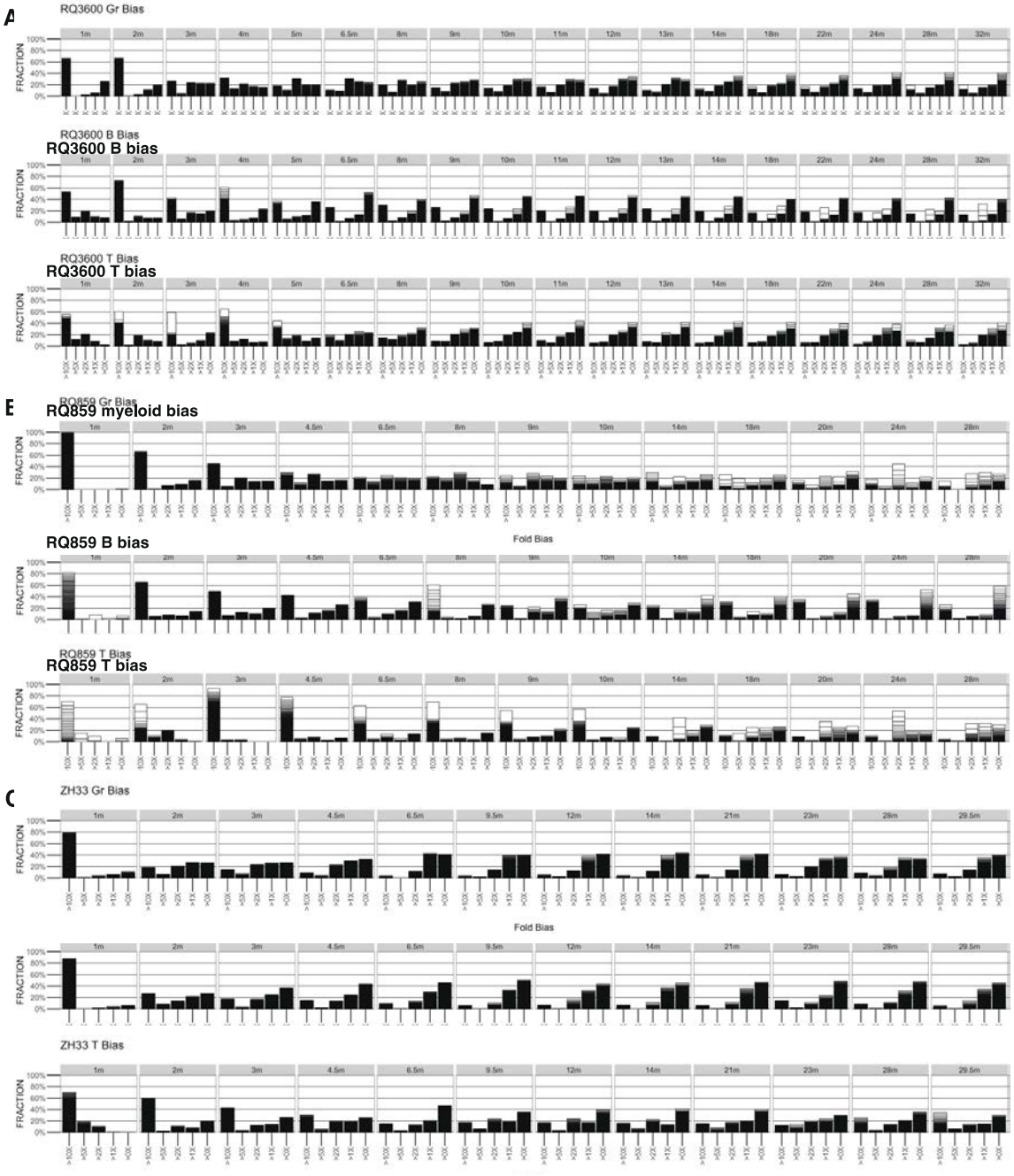
**Supplementary Figure 2**

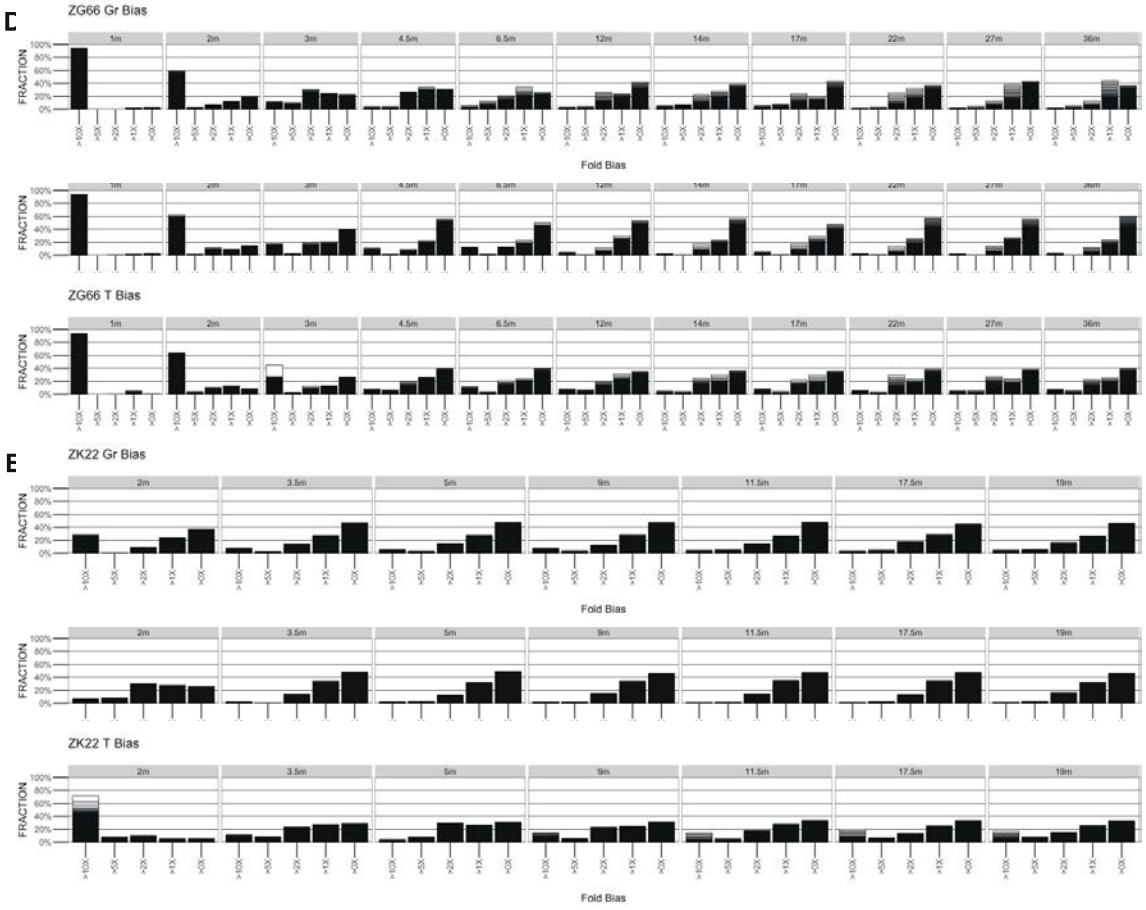



**Supplementary Figure 2. Hematopoietic reconstitution following autologous transplantation**

Absolute total number of granulocytes, monocytes, T, and B cells (GFP+ and GFP-) were determined in the peripheral blood of autologously transplanted aged and young animals at the indicated time points post-transplant. Counts are expressed as cells of interest per uL peripheral blood.

Supplementary Figure 3

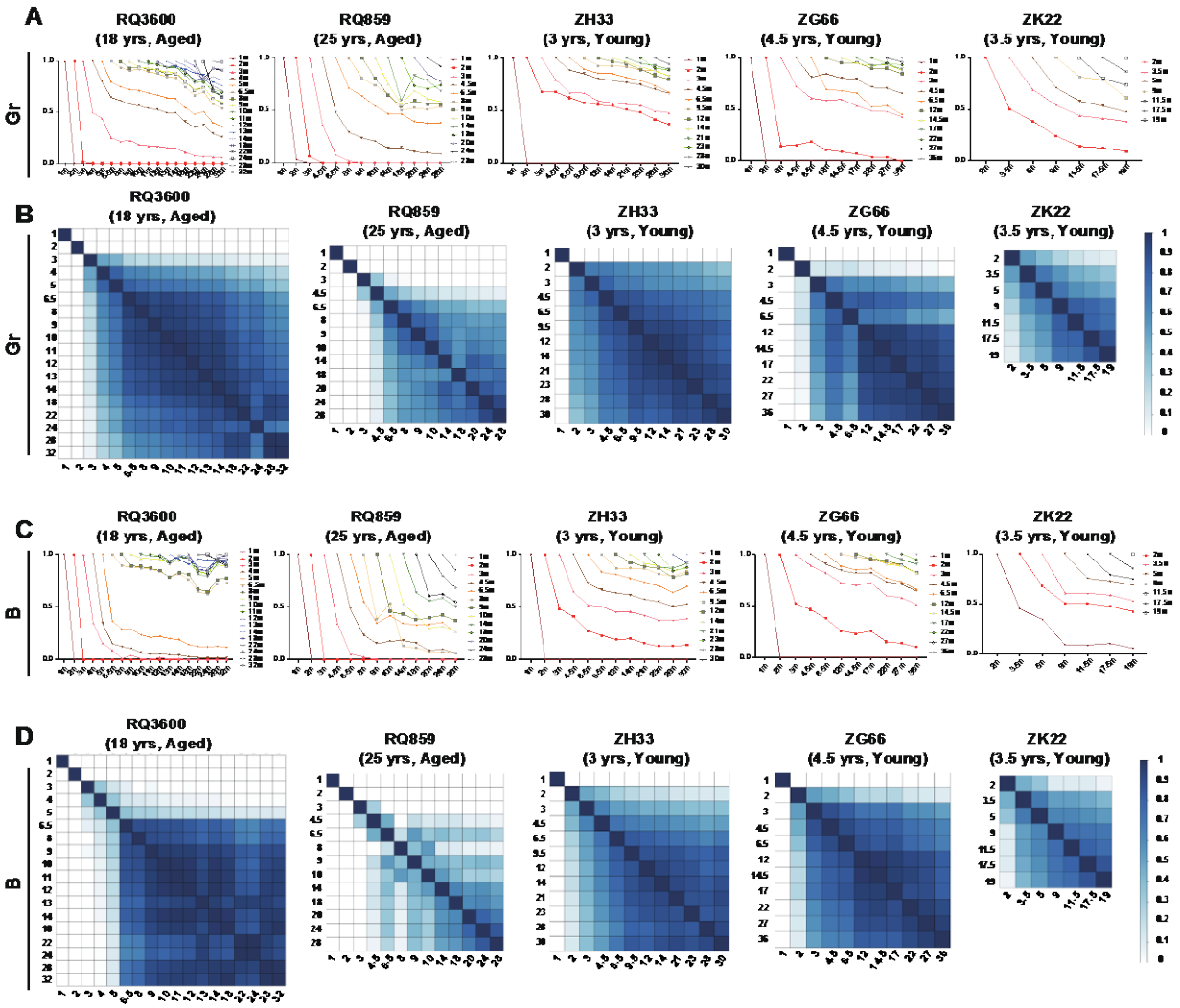





**Supplementary Figure 3. Long-term tracking of non-biased, 1, 2x, 5x, 10x T, B, or myeloid-biased clones**

**A-E)** Histograms showing total contributions from clones that are non-biased, 1x, 2x, 5x, or 10x biased towards each lineage versus all other lineages are plotted on the x axis as stacked bars for RQ3600 (A), RQ859 (B), ZH33 (C), ZG66 (D), and ZK22 (E) at each time point. The clones in each bias bin are stacked largest to smallest, and horizontal lines across the bar delineate individual clones. The y axis shows the total contribution in each bias "bin" of clones. Histograms for T cells, B cells, and Granulocytes (to represent myeloid) are shown.

**Supplementary Figure 4**




**Supplementary Figure 4. Stability of clonal reconstitution in Gr/B lineages**

**A-E)** Pearson correlation coefficients comparing barcode contributions at a certain time point post-transplantation and to all subsequent time points are plotted as individual lines for Gr (A) and B cells (C) as a measure of clonal stability over time. Each time point is shown as a different colored line. Pearson correlations are also plotted with a blue to white color scale for Gr (B), B cells (D).