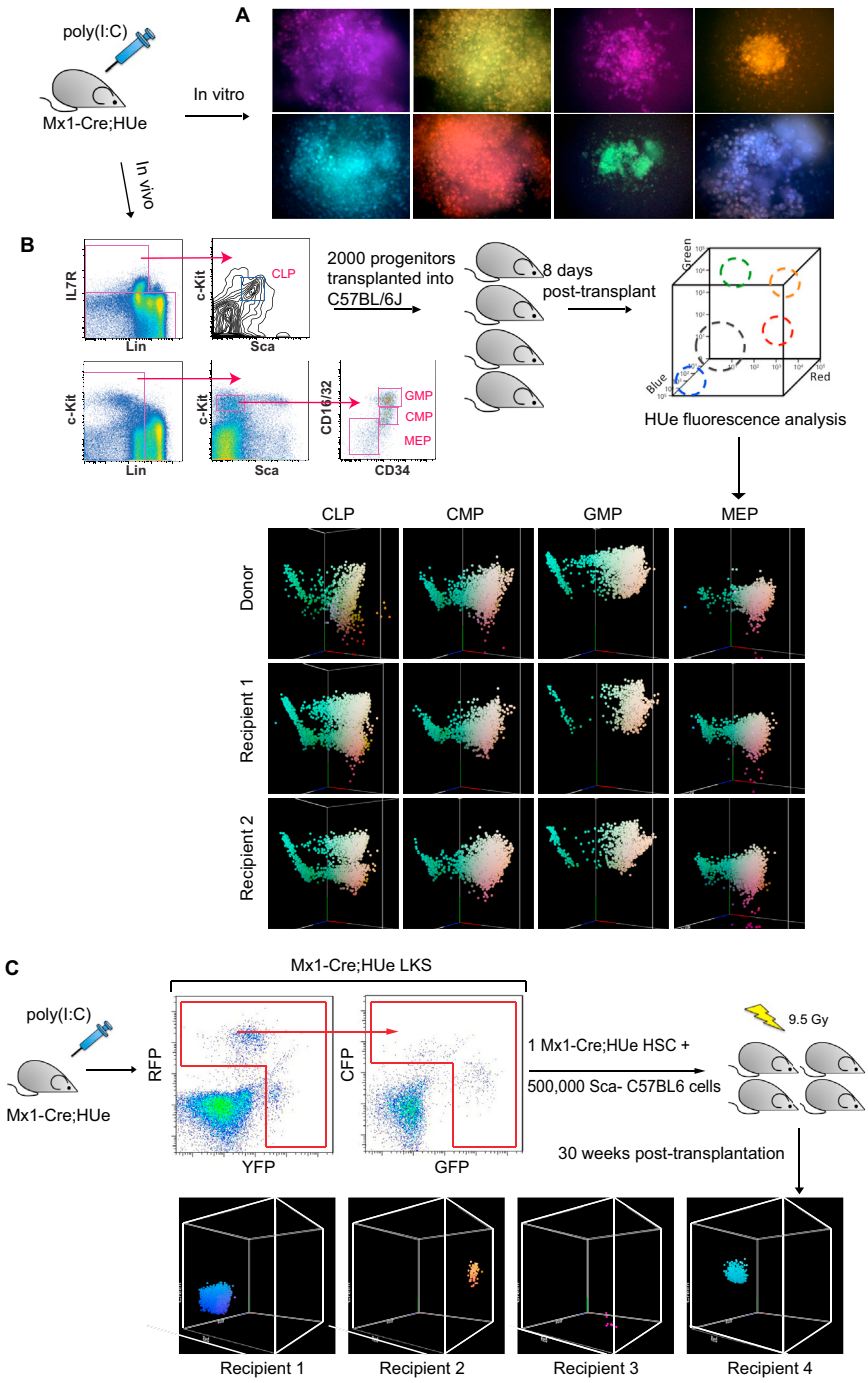

Figure S1. Faithful In Vivo Propagation of Fluorescence over Generations Enables Clonal Tracking, Related to Figure 1

(A) Demonstration of fluorescence fidelity in vitro. Bone marrow cells harvested from induced Mx1-Cre;HUe were plated at low concentration in methycellulose-containing medium for single cell derived hematopoietic colony to emerge and imaged under fluorescent microscope. Uniformity in fluorescence in individual colonies showed that color was consistent over generations of cell division in vitro.

(B) Demonstration of fluorescence fidelity in vivo. Bone marrow cells harvested from induced Mx1-Cre;HUe were flow sorted to isolate CLP, CMP, GMP, and MEP populations. Two thousand cells of each population were intravenously transplanted into each of 5 sublethally irradiated C57BL/6J recipients. Spleens of recipient mice were harvested 8 days post-transplantation, and cells were subjected to flow cytometric analysis of HUe fluorescence. Endogenous HUe fluorescence emanating from cells was plotted in a 3 dimensional graph with x axis (tDimer2 = red fluorescence), y axis (Cerulean = blue fluorescence), and z axis (EYFP = green fluorescence) representing increasing fluorescent intensities in log scale. Recipient mice that received the same batch of donor cells exhibited a HUe fluorescent profile nearly identical to the donor cells injected into them.

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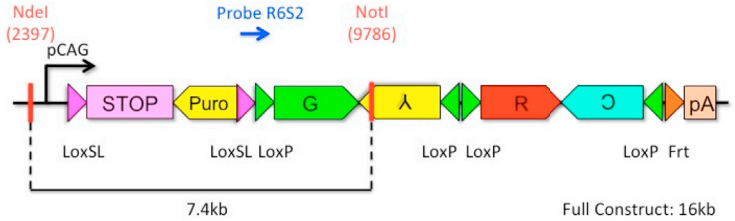
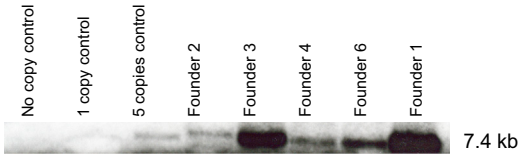
(C) We performed single cell transplantation to further confirm color fidelity in vivo. Single HSCs (Lineage^{Lo}cKit^{+}Sca^{+}CD48^{-}CD150^{+}), each carrying its own unique HUe fluorescent signature, were sorted from induced Mx1-Cre;HUe donors. Each of 214 lethally irradiated C57BL/6J mice was transplanted with a single HUe fluorescent HSC in combination with 500,000 Sca^{-} C57BL/6J bone marrow support cells. At 30 weeks post-transplantation, mice engrafted with fluorescent cells revealed a tight clone emanating from the single cell transplanted.

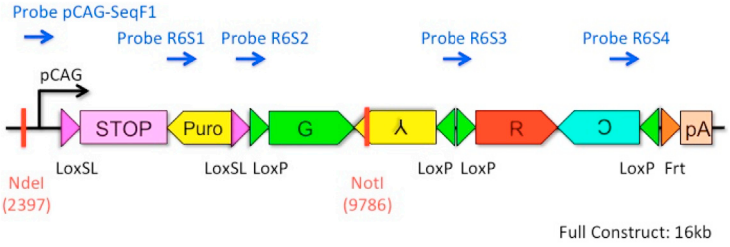
A Southern blot probes to detect number of copies of transgene inserted into the mouse genome:



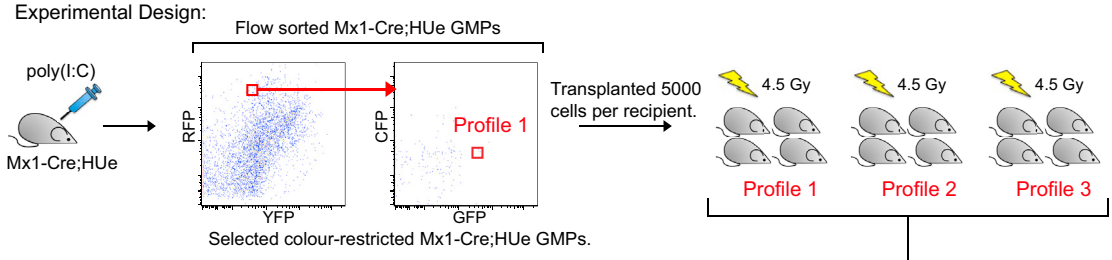
Southern blot detected multiple copies of transgene inserted into the mouse genome in different founder lines:



B DNA Fingerprinting with probes to detect random genomic rearrangement in the presence of Cre:



Experimental Design:



Harvested spleen DNA at day 11 post-transplantation.

DNA Fingerprinting for transgene rearrangement.

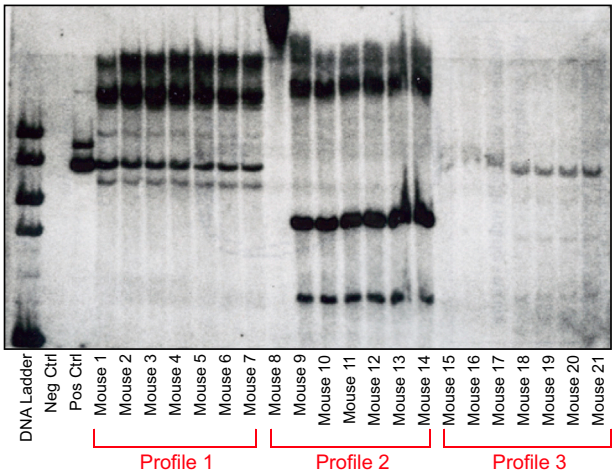


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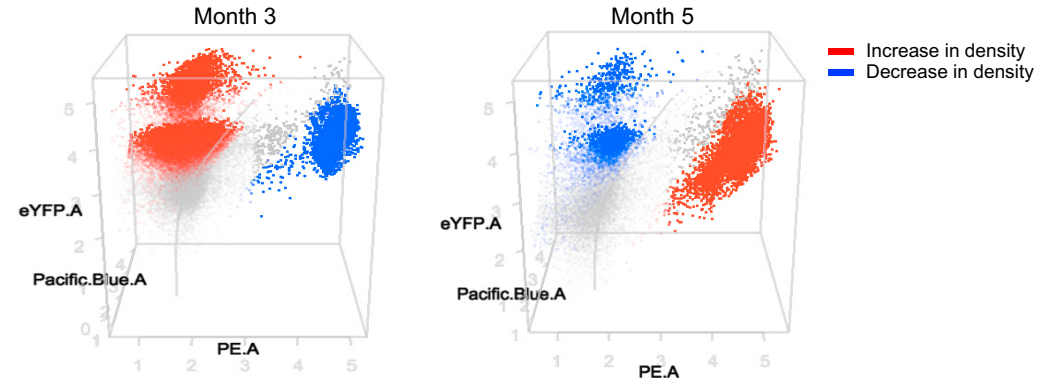
Figure S2. Genetic Confirmation of HUe Fluorescence as a Clonal Marker, Related to Figure 1

(A) A schematic representation of Southern blot probe design to detect the number of transgene copies inserted into the genome. Southern blot detected multiple copies of transgene inserted into the mouse genome in different founder lines.

(B) A schematic representation of DNA fingerprinting probe design to detect random genomic rearrangement in the presence of Cre. To genetically prove that a fluorescent cluster of a defined size was clonal, we sorted HUe colored GMPs from induced Mx1-Cre;HUe mice using a restricted gate drawn on the fluorescent intensity plots during cell sorting. Five thousand GMPs of restricted HUe fluorescence were transplanted into each of seven sublethally irradiated C57BL6/J mice. We sorted GMPs of three different HUe colors and transplanted into 21 mice in total. Eleven days after transplantation, DNA was extracted from whole spleen of each recipient and subjected to DNA fingerprinting for identification of transgene rearrangement, using a set of probes that bind to different regions of the transgene.

A Flow cytometric measurement of HUe clonal fuorescence from bone marrow aspirates at 5 and 10 months old



B Quantification of HUe clonal changes from 5 to 10 months old by custom-designed MClust R program

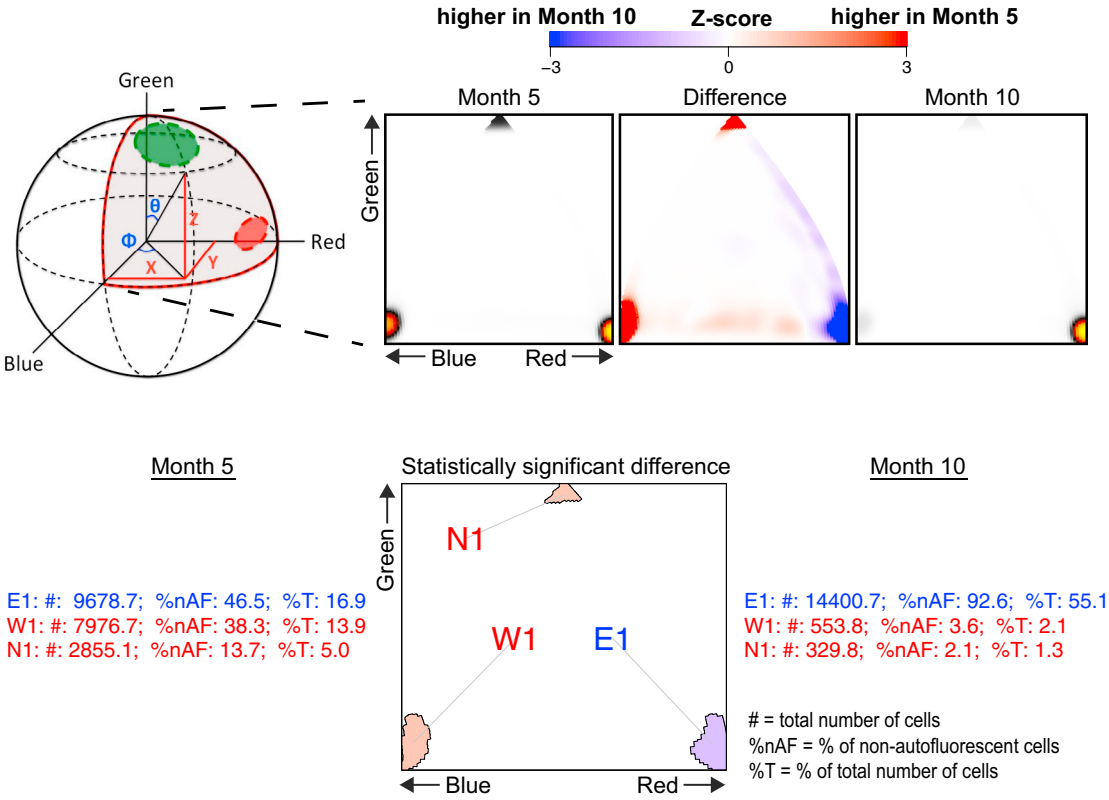


Figure S3. Quantification of Hematopoietic Clonal Changes under Homeostatic Conditions—Mouse #8, 5 to 10 Months Old, Related to Figures 2, 4, 5, and 7

(A) A Mx1-Cre;HUe mouse was induced for endogenous HUe fluorescence with pIpC at one month old. Bone marrow aspirates were obtained from tibiae at 5 and 10 months old respectively. HUe fluorescence in total bone marrow cells was projected in spherical graph with x axis (tDimer2), y axis (Cerulean), and z axis (EYFP) representing increasing fluorescent intensities in log scale. Clones that showed changes are highlighted with colors: red represents an increase in density, while blue represents a decrease in density in comparison to the other time point.

(B) To quantify the pattern changes between the two time points, HUe fluorescence in 3D space was projected into two dimensional plots using sinusoidal projection: 5 months old (on left) and 10 months old (on right), and the difference plot in between. In the difference plot, red represents a decrease in cell density at the indicated HUe fluorescence at 10 months old, whereas blue represents an increase in cell density at 10 months old. White contour line indicates statistically significant changes with a Z-score of −3 to 3. Clones that were statistically different between the two time points were summarized in the panel below. For each clone, we scored the change in absolute number of cells, the percentage of non-autofluorescent cells, and the percentage of the total number of cells.

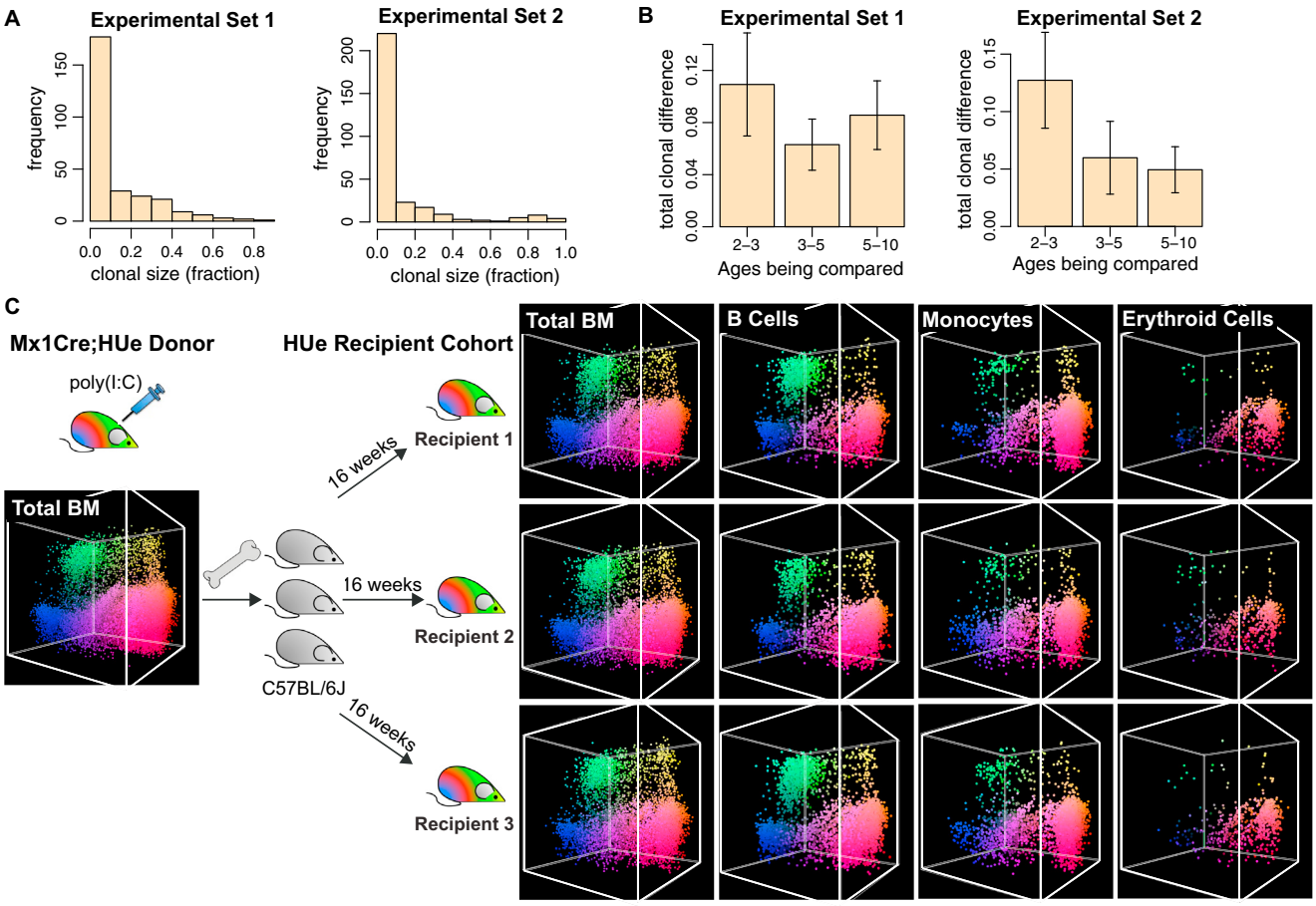


Figure S4. Hematopoiesis Is a Composite of Dissimilar Clones with Stereotypic Behavior, Related to Figures 2 and 3

(A) Distribution of clone sizes (measured as fraction of total cells) is shown for the two sets of mice.

(B) A total fraction of cells affected by shifts in the clonal composition between the adjacent time points is shown. Whiskers show 95% ~~Poisson~~ confidence interval.

(C) Illustrated is another example of HUe recipient cohort. Endogenous fluorescence activated Mx1-Cre;HUe mice were used as donors. Bone marrow cells from multiple Mx1-Cre;HUe donors were pooled as one mixture and flow sorted to isolate HSPCs (Lineage^{Lo}Sca^{+}cKit^{+}). HSCs with random endogenous fluorescence were mixed with support cells from C57BL/6J and transplanted into each of 20 lethally irradiated C57BL/6J recipients. After sixteen weeks of reconstitution, the recipients showed high consistency in clonal pattern including proliferation, fluorescence, and lineage characteristics in all hierarchy of hematopoietic cell types. HUe clonal fluorescent patterns of B cells, monocytes, and erythroid cells in multiple recipients are shown, illustrating consistency among the recipients and the distinction between different cell compartments.

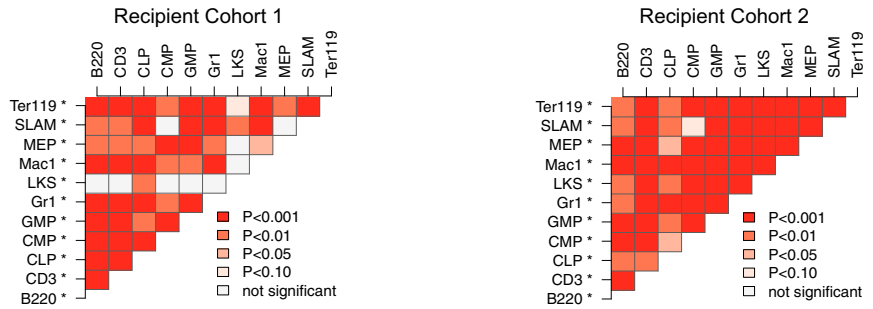
A Clonal consistency within each cell type across multiple mice in a HUe recipient cohort

cell	P-value
B220	8.7e-08
CD3	3.0e-08
CLP	7.1e-07
CMP	3.9e-04
GMP	1.9e-02
Gr1	1.1e-08
LKS	1.7e-07
Mac1	2.1e-07
MEP	7.1e-04
SLAM	2.9e-12
Ter119	1.3e-12

B Clonal pattern of each cell type is uniquely distinct from others. Comparing correlation coefficients within each cell type against other cell types in the same army.

cell/dataset	Cohort 1	Cohort 2	combined
B220	5.0e-07	3.7e-01	9.8e-26
CD3	3.3e-06	1.5e-12	5.4e-17
CLP	1.2e-08	1.7e-11	2.0e-23
CMP	1.1e-15	2.7e-07	4.6e-17
GMP	1.5e-12	3.4e-12	2.5e-31
Gr1	1.5e-10	2.2e-03	3.6e-41
LKS	2.4e-15	2.1e-04	3.0e-20
Mac1	1.9e-10	1.3e-02	8.4e-32
MEP	4.3e-13	8.1e-08	1.3e-31
SLAM	2.3e-15	1.4e-15	6.0e-43
Ter119	1.0e-03	4.2e-04	9.0e-01

C Clonal differences among cell types are consistant within a given HUe recipient cohort



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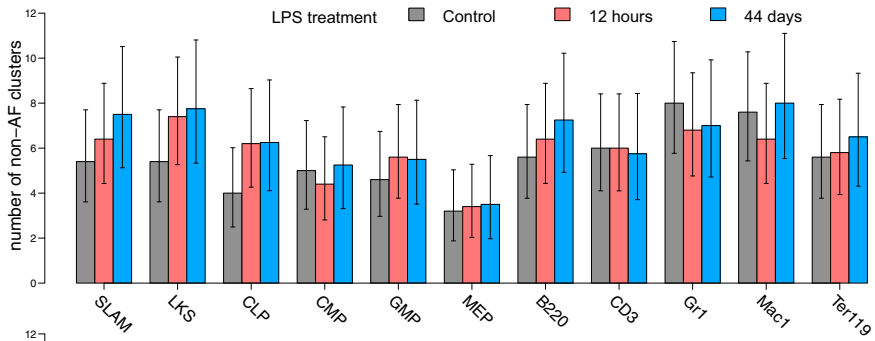

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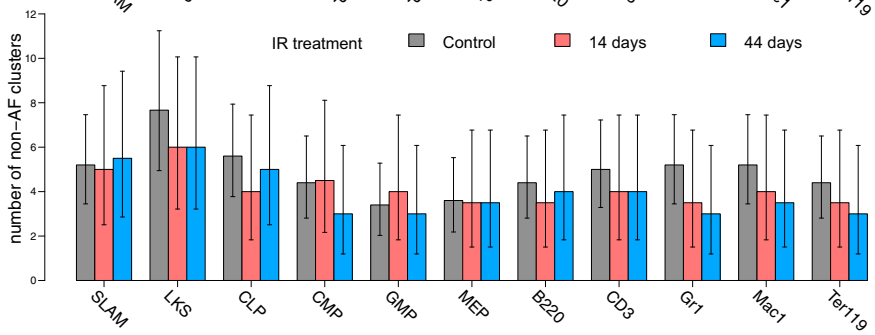

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Figure S5. Hematopoietic Functional Heterogeneity Is Preserved across All Mice in Any Given HUe Recipient Cohort, Related to Figures 3 and 4

(A) Individual hematopoietic cell populations show significant clonal consistency across members within a HUe recipient cohort. Clonal pattern of a hematopoietic cell type (e.g., B220) was highly similar across multiple mice in any given HUe recipient cohort. Table shows Wilcoxon test p values for each hematopoietic cell type, by comparing correlation coefficients of the same cell type across mice within the same recipient cohort.

(B) Clonal pattern of each cell type is uniquely distinct from others. We compared correlation coefficients within each cell type against other cell types in the same recipient cohort. Table shows Wilcoxon test p values comparing correlations of one cell type (e.g., B220) versus other cell types (e.g., CD3). Data of two independent HUe recipient cohorts are shown. The largest p value (combined Wilcoxon tests) was 6e-43.

(C) Clonal differences among cell types are consistent within a HUe recipient cohort. We interrogated the pattern differences between any two cell types (*i.e*. CLP versus B220), and asked whether this change in clonal pattern was consistent among all mice within a recipient cohort. The triangular matrices summarize the statistical significance of the clonal changes in different cell population comparisons. The asterisk on the row label indicates that when testing for fluorescence spectrum changes of a given cell population against the others, the differences between the mice were significantly smaller than the differences between cell population pairs. The largest p value (Wilcoxon test) was 1e-13.

(D, E). Effect of LPS and IR stress on cluster counts. The barplots show total number of clusters observed for different compartments in control and post-perturbation mice for LPS (D) and irradiation (E) perturbations. x axis lists the groups of hematopoietic cell types analyzed at 12 hr and 44 days post-treatment compared to mock treatment controls. y axis indicates the number of non-autofluorescent clusters per sample. While inter-mouse variation in cluster numbers is substantial (whiskers give 95% Poisson confidence interval), analysis across different compartments using Poisson GLM shows statistically significant deviations. Specifically, compared to control, LPS treatment led to significant increase of cluster numbers in the stem cell/progenitor populations (SLAM+LKS+CLP+GMP, p value 0.038 for 12 hr and 0.021 for 44 day samples). On day 44, the increase is statistically significant even downstream effector compartments are considered (p value 0.0361). In contrast, IR treatment shows reduction of cluster numbers (p value 0.071).

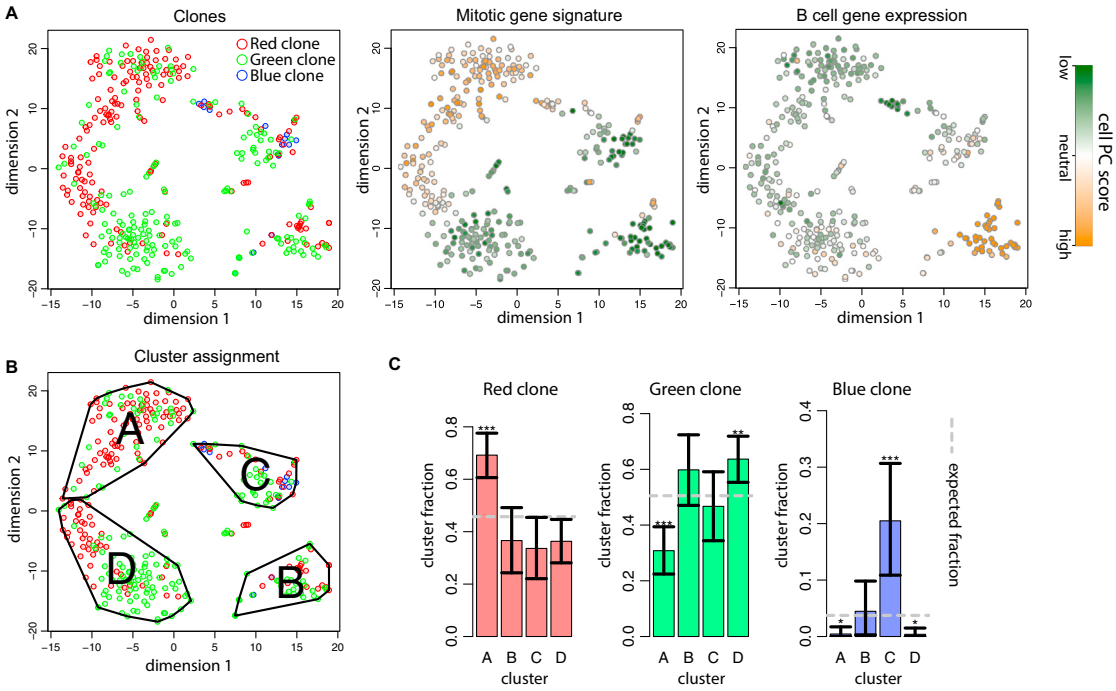

Figure S6. Transcriptional Heterogeneity within and between Clones as Illustrated by Single-Cell RNA-Seq Analysis, Related to Figures 5, 6, and 7

Single HSCs (Lineage^{Lo}cKit^{+}Sca^{+}CD48^{-}CD150^{+}) belonging to individual clones were flow sorted from a pIpC induced Mx1-Cre;HUe mouse and subjected to single-cell RNA-seq analysis.

(A) tSNE visualization of transcriptional heterogeneity, with cells colored according to (from left to right plot) the HUe clone they belong to (red, green or blue clones), intensity of the mitotic signature (orange – high mitotic expression activity, green – low), and intensity of B cell like signature. The distribution of clones showed notable bias toward particular transcriptional states, however the cells of both large clones (red and green) can be found throughout the transcriptional space, indicating that despite overall transcriptional and phenotypic bias, substantial intra-clonal transcriptional variability was present.

(B) The plot shows transcriptional cluster definitions. Clusters A-D describe key subpopulations, with the small erythroid-like and neutrophil-like groups in the center omitted.

(C) Plots show tests of distribution of different HUe clones within different transcriptional clusters. Each subplot tests distribution of a particular HUe clone (red, green, blue). The clusters were defined by x axis, and the y axis represents the fraction of each transcriptional cluster taken up by a given clone. The dashed horizontal gray line shows the fraction of the transcriptional cluster the HUe clone was supposed to account for based on clone's overall frequency. The bars show observed fraction, with the whiskers providing 95% CI. The stars indicate statistical significance (*p = 0.05, **p = 0.01, ***p = 0.001).

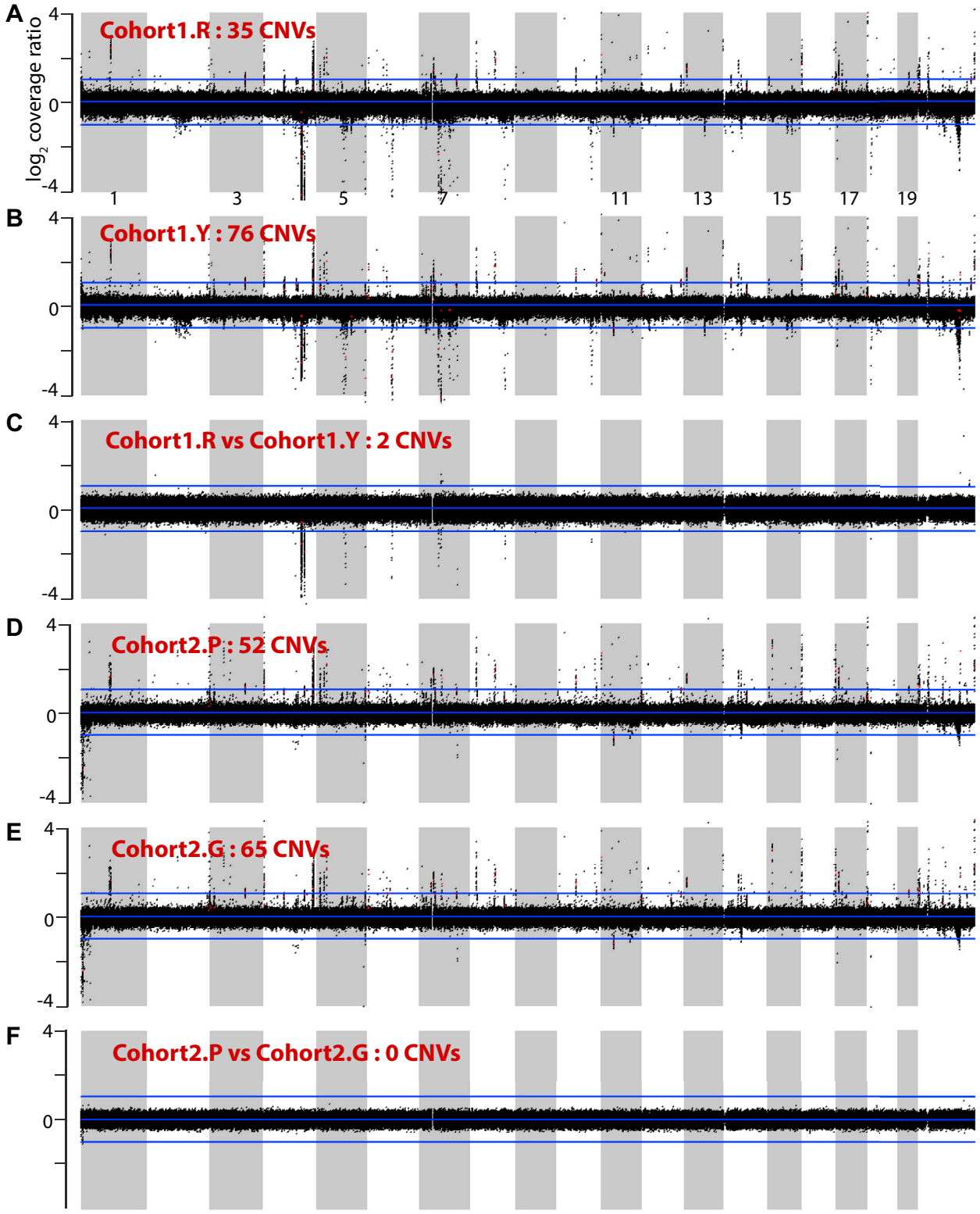

Figure S7. Analysis of Genomic Differences between the Clones, Related to Figures 5, 6, and 7

The CNV profiles obtained from the WGBS data for each clone are shown. Black and red dots represent $\log_2$ copy ratios of bins and CNV segments, respectively. The blue lines represent log2 copy ratios of zero, −1 and +1.

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(A and B) Control-free CNV profiles for the R and Y clones from Cohort1. Many CNVs were detected within each clone (see titles), though almost all occur in both clones.

(C) CNV profile resulting from direct comparison of Cohort1.R and Cohort1.Y clones. Two closely spaced CNVs reported on chromosome 4 appeared to be WGBS artifacts: the first deletion was also called in both Cohort1.R and Cohort1.Y with almost identical breakpoints by the control-free CNV calling method (A, B), indicating that the clone-specific deletion was likely a calling artifact. The adjacent large CNV (~31.6 Mbp) was not called in either Cohort1.R or Cohort1.Y clone. Instead, the region consisted of four smaller CNV segments with similar breakpoints and $\log_2$ ratios in both clones, indicating that the large deletion was unlikely to be a genuine clone-specific deletion and caused by an erroneous CNV segmentation.

(D–F) Analogous control-free and direct comparison plots for Cohort2.P and Cohort2.G clones. In this case, CNV pattern appears to be identical between clones with no notable deviations in the direct comparison. In summary, we found no convincing evidence of clone-specific CNVs despite the reasonable sensitivity of our CNV detection method (see the STAR Methods).