**Supplementary Figure S1: Sorting scheme of HSPC subpopulations based on their surface marker profile.** Bone marrow cells were harvested from male donor animals and after MACS-based lineage-depletion stained with the respective antibodies (Supplementary Table 1) against the markers ScaI, cKit, CD34 and CD150.        HSCs were defined as ScaI+ cKit+ CD150+ CD34-,
            MPPs as ScaI+ cKit+ CD150+ CD34+,
            CMPs as ScaI- cKit+ CD150- CD34+ and
            CLPs as ScaI/ cKit Int CD150- CD34-.
Colors of the plots show parental gate in the former plot and resemble the color scheme of HSPC subpopulation used for visualization of the stained population in the subsequent plots.

**Supplementary Figure S2: Gating strategy to determine the frequency of initially marked HSPC in mature blood cell populations.**

Single suspensions from spleen were first stained with antibodies against CD3e (for T cells), against B220 (for B cells) from the lymphoid compartment and against CD11b (for monocytes/macrophages and against CD11b and Ly6G (for granulocytes). After determination of the morphological cell population (P1), subsequent gating on the respective stained mature cell populations showed the frequency of the marked cells. Shown is one representative animal from the O-O group.
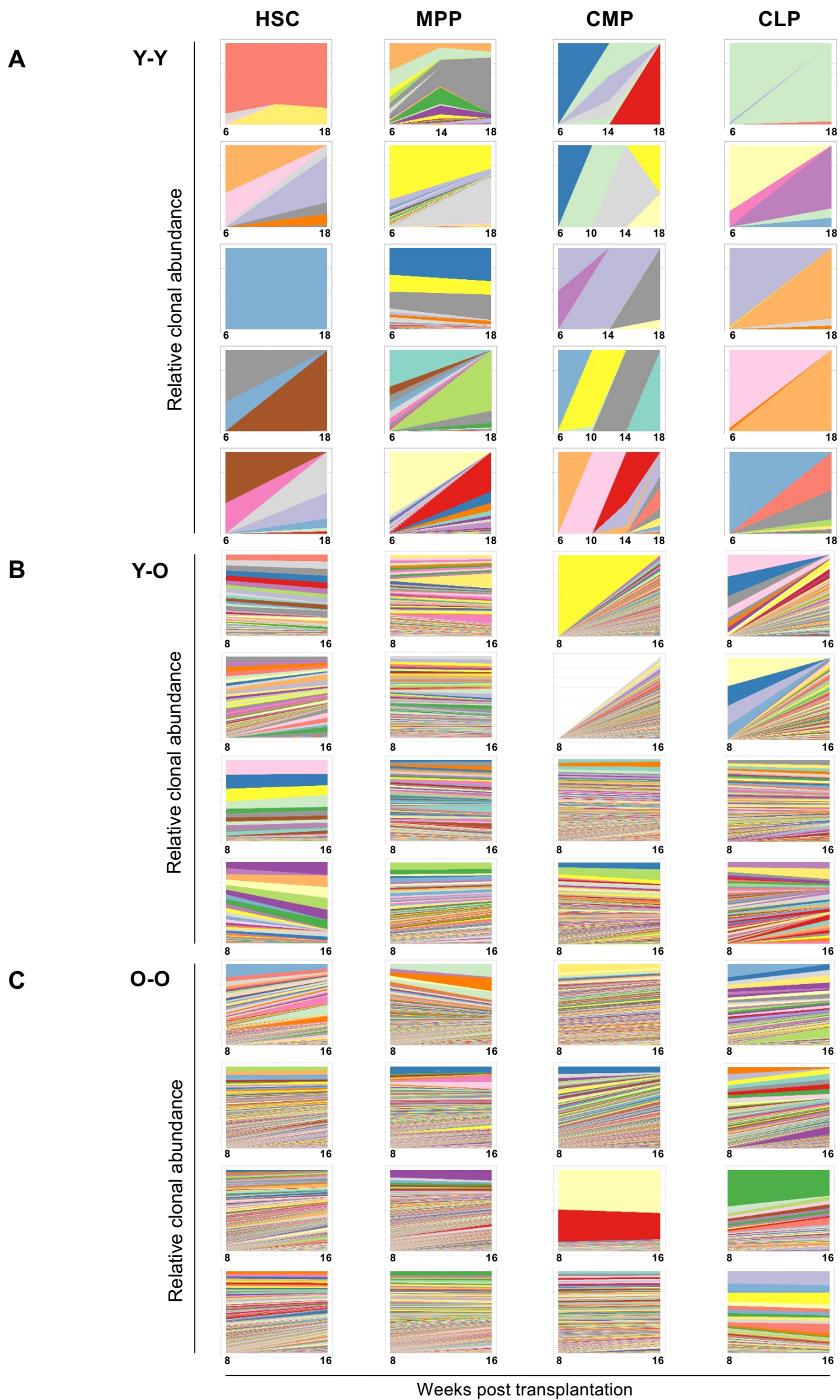
**Lymphoid cells**

**Myeloid cells**
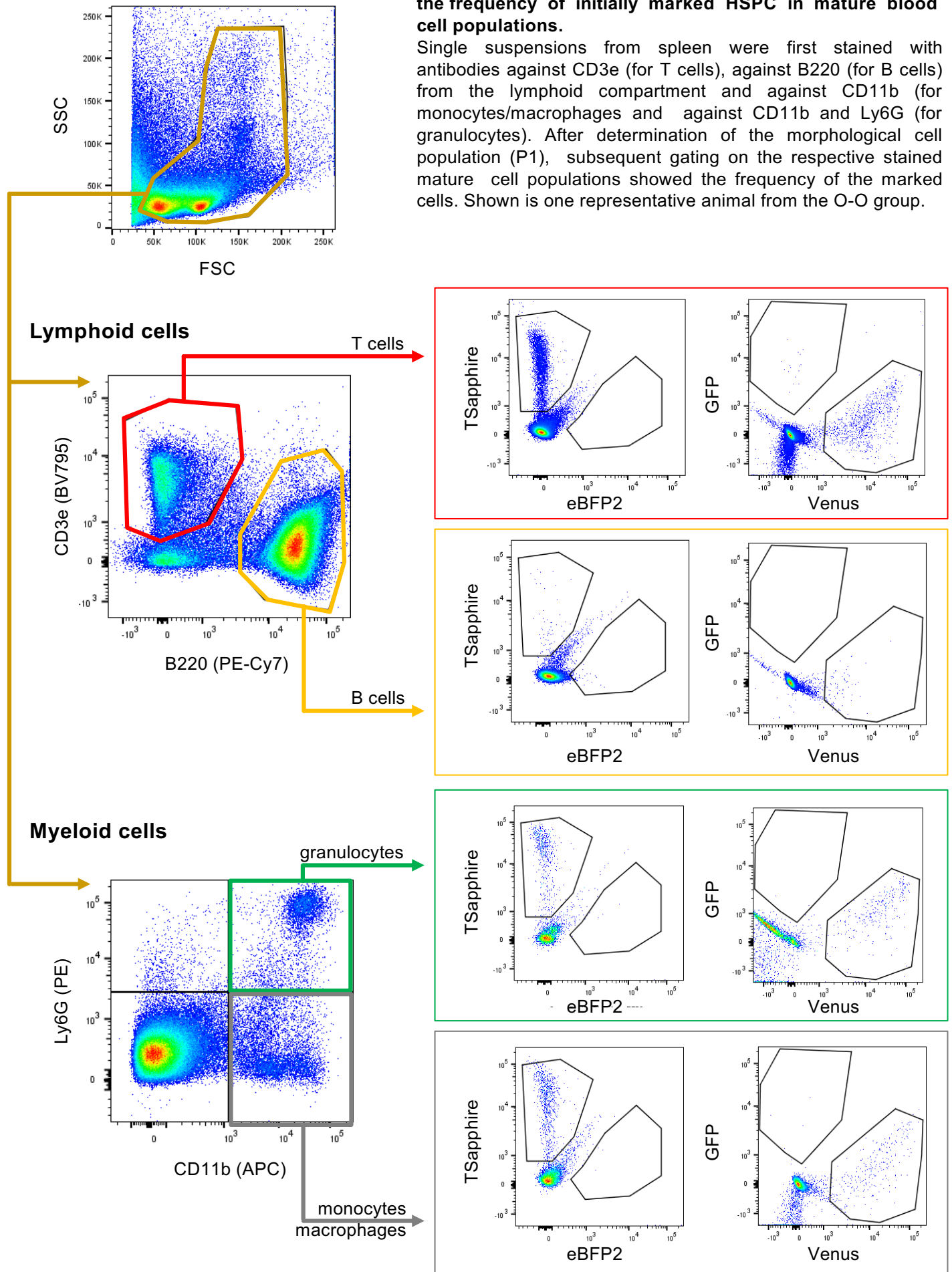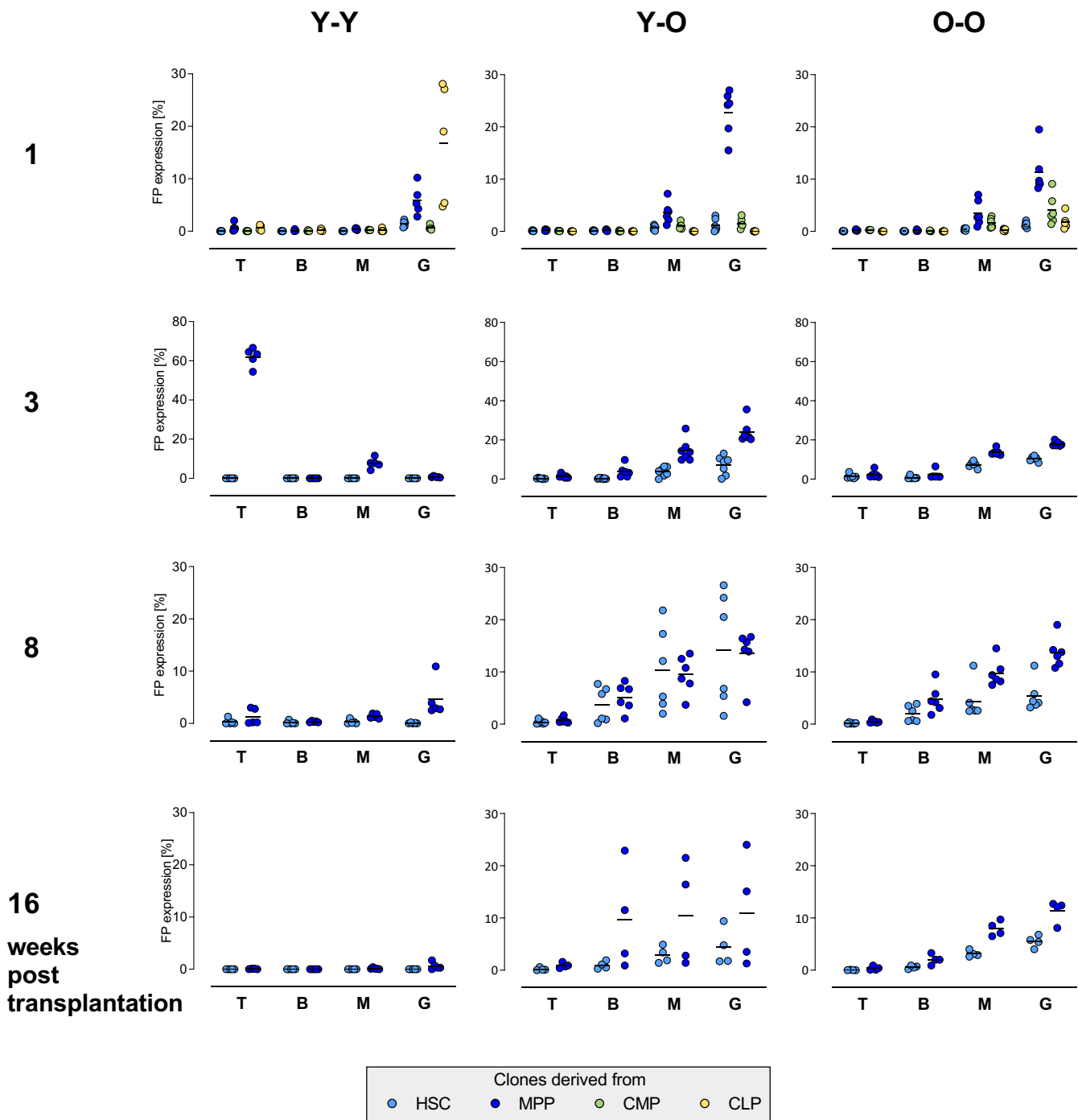
**Supplementary Figure S3: Contribution of marked cell populations in mature blood cell compartments**
FP expression was measured in the T cell (T; CD3), B cell (B; B220), monocyte/macrophage (M; CD11b) and the Granulocyte (G; CD11b/Ly6G) compartment by FACS analysis. The spleen was mostly comprised of HSC- and MPP-derived cells. CMP-derived cells were solely detectable 1 week after transplantation. CLP-derived cells were barely detectable. No data available for CMP- and CLP-derived cells 3, 8 or 16 weeks post transplantation.
Light-blue dots: HSC-derived clones, dark-blue dots: MPP-derived clones, green dots: CMP-derived clones and yellow dots: CLP-derived clones.

|  | | HSC | MPP | CMP | CLP |
|---|---|---|---|---|---|

**A** Y-Y

**B** Y-O

**C** O-O

Relative clonal abundance

Weeks post transplantation

4

**Supplementary Figure S4: Contribution of unique clones in peripheral blood.**
Consecutive peripheral blood samples were harvested from animals of the Y-Y, Y-O and O-O experimental groups throughout the experimental course. For the Y-Y group, up to four samples were taken on week 6, 10, 14 and 18 post transplantation. For the Y-O and O-O group, two samples at 8 or 16 weeks post transplantation were taken. Depending on the amount of obtained blood, the concentration of the extracted DNA was too low to perform PCR based barcode-retrieval. Therefore, we omitted those data points from the plots.
Relative clonal abundancies are depicted as a function of the measurement time points. Each clone is shown with one individual color. An overall lower abundancy of clones was observed in the Y-Y experimental part, while Y-O and O-O experimental parts were more heterogeneous. Each row of plots represents one representative animal from each group.
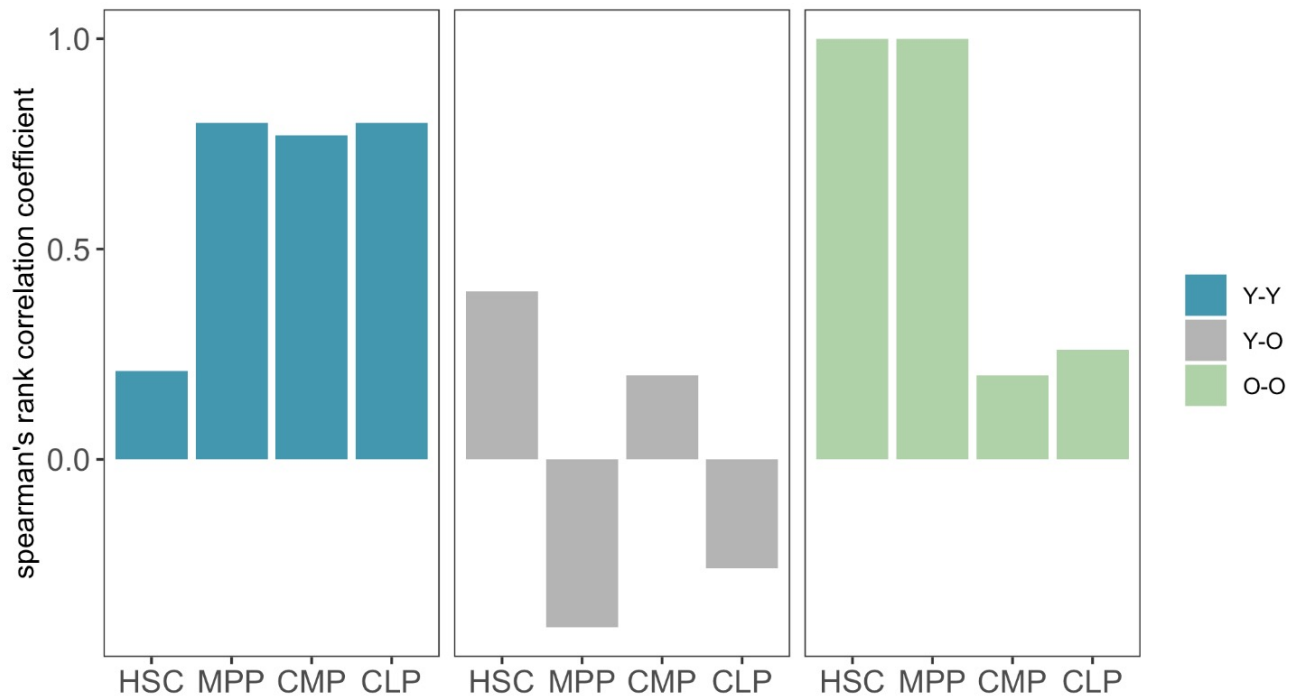
**Supplementary Figure S5: Spearman's correlation coefficients to quantify the correlation between flow cytometry and barcode data**

The mean values of the percentage of fluorescent positive cells taken from spleen (Fig. 4) at each time point and for each experimental setting and the respective mean barcode counts (Fig. 5) were used to calculate the Spearman's rank correlation coefficients to quantify the interrelation of the two measurement methods.

**Supplementary Table S1: Antibodies used for fluorescence-activated cell sorting of HSPC populations and final analyses.**

| Name | Clone | Company | Application |
|------|-------|---------|-------------|
| anti CD16/32 | 93 | Biolegend | Sort of HSPC subpopulations/ Final Analyses |
| PE-Cy7 anti ScaI | E13-161.7 | Biolegend | Sort of HSPC subpopulations/ Final Analyses of BM populations |
| BV421 anti cKit | 2B8 | BD Biosciences | Sort of HSPC subpopulations |
| Pacific Blue anti cKit | 2B8 | Biolegend | Sort of HSPC subpopulations |
| APC anti CD150 | TC15-12F12.2 | Biolegend | Sort of HSPC subpopulations |
| PE anti CD34 | RAM34 | BD | Sort of HSPC subpopulations |
| APC-Cy7 anti cKit | 2B8 | Biolegend | Final Analyses of BM populations |
| PE anti CD150 | TC15-12F12.2 | Biolegend | Final Analyses of BM populations |
| APC anti lineage | diverse | BD (558074) | Final Analyses of BM populations |
| APC anti lineage | diverse | R&D Systems (FLC001A) | Final Analyses of BM populations |
| APC anti CD48 | HM48-1 | Biolegend | Final Analyses of BM populations |
| PE-Cy7 anti B220 | RA3-6B2 | Biolegend | Final analyses of mature blood cells in spleen |
| BV795 anti CD3e | 145-2C11 | BD | Final analyses of mature blood cells in spleen |
| PE anti Ly.6G | 1A8 | Biolegend | Final analyses of mature blood cells in spleen |
| APC anti CD11b | M1/70 | Biolegend | Final analyses of mature blood cells in spleen |

## Supplementary Table S2: Oligonucleotides used for Barcode library construction and analysis.

N positions indicate sites for random insertion of one of the four nucleotides during production, x indicates phosphothioate bond and BHQ1 stands for Black Hole Quencher 1.

| Internal name | Sequence (5'-3') | Application |
|---|---|---|
| Poly-GFP-BC-fw | GGTGCATCTAGAACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNACTNN CGANNCTTNNCGANNCTTNNGGANNCTANNACTNNCGANNCTTNNCGANNCTT NNGGANNCTANNACTNNCGANNCTCGAGGTGCACTATG | Oligo for GFP-BC library construction |
| Poly-Venus-BC-Fw | GGTGCATCTAGAACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNCGANN AGANNCTTNNCGANNCTANNGGANNCTTNNCGANNAGANNCTTNNCGANNCTA NNGGANNCTTNNCGANNAGANNCTCGAGGTGCACTATG | Oligo for Venus-BC library construction |
| Poly-Cerulean-BC-Fw | GGTGCATCTAGAACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNCAGNN ATCNNCTTNNCGANNGGANNCTANNCTTNNCAGNNATCNNCTTNNCGANNGGA NNCTANNCTTNNCAGNNATCNNCTCGAGGTGCACTATG | Oligo for T-Sapphire-BC library construction |
| Poly-Cherry-BC-Fw | GGTGCATCTAGAACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNCTANN CAGNNCTTNNCGANNCTANNCTTNNGGANNCTANNCAGNNCTTNNCGANNCTA NNCTTNNGGANNCTANNCAGNNCTCGAGGTGCACTATG | Oligo for BFP-BC library construction |
| 32BC-Poly-fw | GGTGCATCTAGAACACTC | Generation of ds BC oligo |
| 32BC-Poly-rev | CATAGTGCACCTCGAG | Generation of ds BC oligo |
| Illu_P2 (43) | GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT | Bridging oligo for sequencing |
| Illu_MPLX35 (164) | CAAGCAGAAGACGGCATACGAGATTCTGAGGTGACTGGAGTTC | Index35 |
| Illu_MPLX36 (165) | CAAGCAGAAGACGGCATACGAGATCCTTGCGTGACTGGAGTTC | Index36 |
| Illu_MPLX37 (166) | CAAGCAGAAGACGGCATACGAGATTGGAGCGTGACTGGAGTTC | Index37 |
| Illu_MPLX38 (167) | CAAGCAGAAGACGGCATACGAGATTCGGGAGTGACTGGAGTTC | Index38 |
| Illu_MPLX39 (168) | CAAGCAGAAGACGGCATACGAGATAAACCT GTGACTGGAGTTC | Index39 |
| Illu_MPLX40 (169) | CAAGCAGAAGACGGCATACGAGAT CTCTAC GTGACTGGAGTTC | Index40 |
| Illu_MPLX41 (170) | CAAGCAGAAGACGGCATACGAGAT CGGCCT GTGACTGGAGTTC | Index41 |
| Illu_MPLX42 (171) | CAAGCAGAAGACGGCATACGAGATCCGGTGGTGACTGGAGTTC | Index42 |
| Illu_MPLX43 (172) | CAAGCAGAAGACGGCATACGAGATCAGCAGGTGACTGGAGTTC | Index43 |
| Illu_MPLX44 (173) | CAAGCAGAAGACGGCATACGAGATAAGTGCGTGACTGGAGTTC | Index44 |
| Illu_MPLX45 (174) | CAAGCAGAAGACGGCATACGAGATCAGGCCGTGACTGGAGTTC | Index45 |
| Illu_MPLX46 (175) | CAAGCAGAAGACGGCATACGAGATGGTAGAGTGACTGGAGTTC | Index46 |
| Illu_MPLX47 (176) | CAAGCAGAAGACGGCATACGAGATCCAGCAGTGACTGGAGTTC | Index47 |
| Illu_MPLX48 (177) | CAAGCAGAAGACGGCATACGAGATGCGCCAGTGACTGGAGTTC | Index48 |
| Illu_MPLX49 (178) | CAAGCAGAAGACGGCATACGAGATGGAACTGTGACTGGAGTTC | Index49 |
| Illu_MPLX50 (179) | CAAGCAGAAGACGGCATACGAGATGCGGACGTGACTGGAGTTC | Index50 |
| Illu_MPLX51 (180) | CAAGCAGAAGACGGCATACGAGATCGAAACGTGACTGGAGTTC | Index51 |
| Illu_MPLX52 (181) | CAAGCAGAAGACGGCATACGAGATCCACTCGTGACTGGAGTTC | Index52 |
| ILL_Dual_P5-01 | AATGATACGGCGACCACCGAGATCTACACAGCTTAGTACACTCTTTCCCTACA CGACGCTCTTCCGATCxT | Dual Index01 |
| ILL_Dual_P5-02 | AATGATACGGCGACCACCGAGATCTACACGCTACTTGACACTCTTTCCCTACA CGACGCTCTTCCGATCxT | Dual Index02 |
| ILL_Dual_P5-03 | AATGATACGGCGACCACCGAGATCTACACCTAGGCACACACTCTTTCCCTACA CGACGCTCTTCCGATCxT | Dual Index03 |
| ILL_Dual_P5-04 | AATGATACGGCGACCACCGAGATCTACACTAGCAGCAACACTCTTTCCCTACA CGACGCTCTTCCGATCxT | Dual Index04 |
| Y Chromosome Fw | ACAAGTTTTGGGACTGGTGACAA | ddPCR Y chromosome |
| Y Chromosome Rv | ACCACGGGACCACACCATAA | ddPCR Y chromosome |
| Y Chromosome-p | TGTCAAGCGCCCCATGAATGCA-BHQ1 | ddPCR Y chromosome |
| LeGOddBCi Fw | CTAGAAAAACATGGAGCAATCACAA | ddPCR illumina sequence |
| ddBCillu Rv | AGCGTCGTGTAGGGAAAGAGTG | ddPCR illumina sequence |
| ddBCIllu-p | CTGAATGATACGGCGACCACCGTCTAG | ddPCR illumina sequence |
| mEpo Chromosome Fw | GCAGGCGGGGTCGCTACTC | ddPCR mEpo |
| mEpo Chromosome Rv | CGCCTGTGCAGATCCGATAA | ddPCR mEpo |
| mEpo Chromosome-p | TTCTGAGGCGCCACTTTTGCAAGACC | ddPCR mEpo |

**Supplementary Table S3: Indirect estimation of the average vector copy number**

Single cells from spleen of transplanted animals (randomly chosen from the respective experimental group) were sorted based on their fluorescent protein expression. Cells were directly used for PCR to amplify the barcode sequence(s) using wpre-fw (5'-GAGGAGTTGTGGCCCGTTGT) and TA98 (5'-GGCTGTCAAACCTCCACTCT) for the first PCR and TA97 (5'-CATCGATACCGTCGACCTC) and TA99 (5'-TCGTTGGGAGTGAATTAGCC) for the second PCR. PCR-products were gelpurified and Sanger sequenced using TA99. Individual vector copy numbers (VCN) were determined as described in Bystrykh et al., 2016. Assuming that the viral integrations per cell are independent events, the total number of integrations per cell (individual vector copy number, VCN) follows a Poisson distribution. Based on the frequency of individual VCN observed among the single cells, we fit such a Poisson distribution to the empirical data for which the parameter lambda corresponds to the mean number of integrations (examples below the table for the HSC derived samples). Based on these fits we can also estimate the fraction of "untransduced cells" which is given as exp(-lambda). Conversely, the fraction of marked cells (i.e. the transduction efficiency) can be calculated as (1 - exp(-lambda)).

| Y-O | | | | O-O | | | |
|---|---|---|---|---|---|---|---|
| **HSC-derived** | | **MPP-derived** | | **HSC-derived** | | **MPP-derived** | |
| **Sample** | **VCN** | **Sample** | **VCN** | **Sample** | **VCN** | **Sample** | **VCN** |
| 1 | 3.0 | 1 | 3.0 | 1 | 2.0 | 1 | 2.0 |
| 2 | 3.5 | 2 | 3.0 | 2 | 2.0 | 2 | 2.0 |
| 3 | 5.0 | 3 | 5.5 | 3 | 2.0 | 3 | 4.0 |
| 4 | 2.0 | 4 | 3.5 | 4 | 1.0 | 4 | 3.0 |
| 5 | 2.0 | 5 | 4.0 | 5 | 1.0 | 5 | 5.5 |
| 6 | 3.0 | 6 | 4.0 | 6 | 1.0 | 6 | 2.0 |
| 7 | 2.0 | 7 | 2.0 | 7 | 4.5 | 7 | 3.0 |
| 8 | 3.0 | 8 | 2.0 | 8 | 1.0 | 8 | 1.0 |
| 9 | 1.0 | 9 | 2.5 | 9 | 1.0 | 9 | 2.0 |
| 10 | 3.0 | 10 | 2.5 | 10 | 1.0 | 10 | 2.0 |
| 11 | 2.0 | 11 | 3.0 | 11 | 2.0 | 11 | 5.0 |
| 12 | 4.0 | 12 | 3.5 | 12 | 2.0 | 12 | 2.0 |
| 13 | 4.0 | 13 | 3.0 | 13 | 2.0 | 13 | 1.0 |
| 14 | 4.0 | 14 | 5.5 | 14 | 2.0 | 14 | 1.0 |
| 15 | 1.0 | 15 | 1.0 | 15 | 3.0 | 15 | 1.0 |
| 16 | 6.0 | 16 | 5.0 | 16 | 2.0 | 16 | 1.0 |
| 17 | 1.0 | 17 | 4.0 | 17 | 4.0 | 17 | 2.0 |
| 18 | 4.0 | 18 | 6.0 | | | 18 | 2.0 |
| 19 | | 19 | 1.0 | | | 19 | 3.0 |
| 20 | | 20 | 1.0 | | | 20 | 1.0 |
| 21 | | 21 | 3.0 | | | | |
| | | | | | | | |
| mean | 2.8 | mean | 3.2 | mean | 1.6 | mean | 2.4 |

Y–O, HSC, mean of poisson = 2.821 , fraction labeled = 0.94
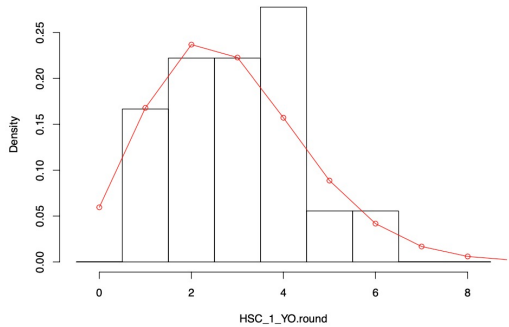
O–O, HSC, mean of poisson = 1.594 , fraction labeled = 0.797

9
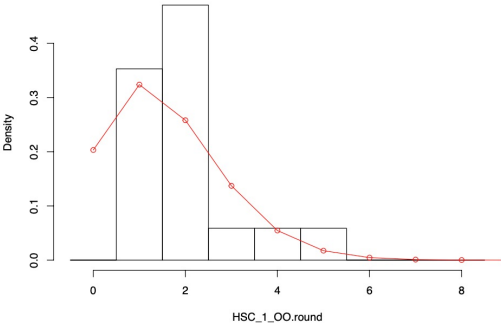
**Supplementary Table S4: Fluorescent protein expressing cells in the mature blood cells**
Flow cytometry data from spleen of the animals (n= 5-7) per group taken after 1,3, 8 or 16 weeks post transplantation. The gating strategy is shown in Supplementary Figure 2. Listed are the mean values and the respective range (min-max).

| | week | ini. subp. | T cells % | T cells min-max | B cells % | B cells min-max | Mono / Macro % | Mono / Macro min-max | Granulocytes % | Granulocytes min-max |
|---|---|---|---|---|---|---|---|---|---|---|
| **Y-Y** | 1 | HSC | 0,0 | 0,0-0,0 | 0,0 | 0,0-0,0 | 0,0 | 0,0-0,0 | 1,4 | 0,7-2,2 |
| | | MPP | 0,1 | 0,0-0,4 | 0,7 | 0,1-2,0 | 0,4 | 0,2-0,6 | 5,9 | 2,8-10,2 |
| | | CMP | 0,1 | 0,0-0,2 | 0,0 | 0,0-0,0 | 0,2 | 0,2-0,3 | 0,7 | 0,3-1,4 |
| | | CLP | 0,2 | 0,0-0,5 | 0,7 | 0,1-1,2 | 0,1 | 0,0-0,7 | 16,9 | 4,7-28,1 |
| | 3 | HSC | 0,0 | 0,0-0,0 | 0,0 | 0,0-0,0 | 0,0 | 0,0-0,0 | 0,0 | 0,0-0,0 |
| | | MPP | 0,0 | 0,0-0,0 | 62,0 | 54,4-66,6 | 7,7 | 4,2-11,6 | 0,7 | 0,4-1,3 |
| | 8 | HSC | 0,2 | 0,0-0,7 | 0,3 | 0,0-1,3 | 0,3 | 0,1-1,0 | 0,1 | 00-0,2 |
| | | MPP | 0,3 | 0,2-0,5 | 1,3 | 0,1-3,0 | 1,4 | 0,9-1,8 | 4,6 | 2,5-10,9 |
| | 16 | HSC | 0,0 | 0,0-0,0 | 0,0 | 0,0-0,0 | 0,0 | 0,0-0,0 | 0,0 | 0,0-0,0 |
| | | MPP | 0,0 | 0,0-0,0 | 0,1 | 0,0-0,1 | 0,1 | 0,0-0,4 | 0,6 | 0,0-1,7 |
| **Y-O** | 1 | HSC | 0,1 | 0,0-0,2 | 0,1 | 0,0-0,2 | 0,7 | 0,1-1,3 | 1,2 | 0,0-3,0 |
| | | MPP | 0,2 | 0,1-0,4 | 0,2 | 0,0-0,4 | 3,6 | 1,2-7,2 | 22,8 | 15,5-27,0 |
| | | CMP | 0,1 | 0,0-0,1 | 0,1 | 0,0-0,2 | 1,0 | 0,5-2,1 | 8,2 | 0,4-3,3 |
| | | CLP | 0,0 | 0,0-0,0 | 0,0 | 0,0-0,0 | 0,0 | 0,0-0,0 | 0,0 | 0,0-0,0 |
| | 3 | HSC | 0,4 | 0,0-0,8 | 0,3 | 0,0-0,7 | 3,7 | 0,0-6,3 | 7,1 | 0,2-13,1 |
| | | MPP | 1,5 | 0,7-3,3 | 3,7 | 1,3-9,8 | 14,5 | 9,9-25,8 | 23,9 | 20,4-35,6 |
| | 8 | HSC | 0,4 | 0,1-1,1 | 3,7 | 0,2-7,7 | 10,4 | 2,0-21,8 | 14,2 | 1,6-26,6 |
| | | MPP | 0,8 | 0,3-1,7 | 5,1 | 1,1-8,3 | 9,5 | 3,7-10,8 | 13,5 | 4,2-16,7 |
| | 16 | HSC | 0,2 | 0,0-0,6 | 1,0 | 0,3-1,9 | 2,9 | 1,4-4,9 | 4,4 | 1,7-9,4 |
| | | MPP | 0,9 | 0,4-1,6 | 9,6 | 0,9-22,9 | 10,5 | 1,4-21,5 | 11,0 | 1,3-24,0 |
| **O-O** | 1 | HSC | 0,0 | 0,0-0,1 | 0,0 | 0,0-0,1 | 0,4 | 0,1-0,6 | 1,3 | 0,6-2,1 |
| | | MPP | 0,2 | 0,1-0,4 | 0,2 | 0,1-0,4 | 3,5 | 0,9-7,0 | 11,3 | 9,1-19,5 |
| | | CMP | 0,3 | 0,2-0,3 | 0,1 | 0,0-0,2 | 1,6 | 0,7-2,9 | 4,1 | 1,4-9,1 |
| | | CLP | 0,0 | 0,0-0,0 | 0,0 | 0,0-0,0 | 0,3 | 0,0-0,5 | 1,8 | 0,5-4,4 |
| | 3 | HSC | 1,4 | 0,4-3,7 | 0,8 | 0,4-2,3 | 7,4 | 4,9-9,5 | 10,4 | 8,3-12,0 |
| | | MPP | 2,3 | 1,1-5,9 | 2,2 | 1,2-6,5 | 13,7 | 12,3-16,8 | 18,0 | 17,0-20,2 |
| | 8 | HSC | 0,2 | 0,1-0,4 | 2,0 | 0,6-3,5 | 4,3 | 2,5-11,2 | 5,4 | 3,2-11,2 |
| | | MPP | 0,5 | 0,3-0,9 | 4,8 | 1,8-9,5 | 9,8 | 7,5-14,5 | 13,7 | 10,8-19,0 |
| | 16 | HSC | 0,0 | 0,0-0,1 | 0,6 | 0,2-0,9 | 3,2 | 2,6-4,0 | 5,5 | 4,0-6,8 |
| | | MPP | 0,4 | 0,1-0,9 | 2,1 | 0,9-3,3 | 8,0 | 6,5-9,7 | 11,3 | 8,1-12,7 |