SUPPLEMENTARY TABLES

Supplementary Table 1A Barcodes used for spiked-in controls

Primary samples	
Barcode	Number of cells
TCATCCTGATGCAAATTGGTGTAACCC	500
AGATCTAGATGGAAAGGGGTCCAACCA	100
GCATCACGATGGAAATAGGTTGAACTT	20
Secondary samples	
Barcode	Number of cells
AGATCCTGATGCAAACGGGTCAAACAC	500
TAATCTCGATCGAAAATGGTAGAACTT	100
AGATCTAGATGGAAAGGGGTCCAACCA	20

Supplementary Table 1B Fraction read abundance for primary transplant data (56 samples)

Library	500 cell control		100 cell control		20 cell control	
	Number of reads	Fraction read abundance	Number of reads	Fraction read abundance	Number of reads	Fraction read abundance
10126	328008	0.9338	20421	0.0581	2820	0.0080
10127	397459	0.9271	25099	0.0585	6155	0.0144
10128	455992	0.9292	28861	0.0588	5881	0.0120
10135	512180	0.9228	38795	0.0699	4080	0.0074
10136	627401	0.9402	33182	0.0497	6725	0.0101
10137	614739	0.9200	46933	0.0702	6525	0.0098
10147	9190	0.9086	805	0.0796	120	0.0119
10149	174144	0.9186	12573	0.0663	2859	0.0151
10150	6445	0.9330	419	0.0607	44	0.0064
10151	57242	0.9097	5104	0.0811	576	0.0092
10152	519248	0.9382	30187	0.0545	4012	0.0072
10153	29369	0.9133	2114	0.0657	673	0.0209
10154	9078	0.9459	364	0.0379	155	0.0162
10155	412287	0.9125	31516	0.0698	8035	0.0178
10156	234543	0.9507	10950	0.0444	1221	0.0049
10157	7544	0.9417	382	0.0477	85	0.0106
10158	504317	0.9210	33111	0.0605	10136	0.0185
10159	7532	0.9443	333	0.0418	111	0.0139
10160	594854	0.9509	27336	0.0437	3370	0.0054
10161	12283	0.9337	670	0.0509	202	0.0154
10162	29690	0.9362	1692	0.0534	332	0.0105
10163	135360	0.9401	6342	0.0440	2279	0.0158
10164	419987	0.9132	33186	0.0722	6746	0.0147
10165	515517	0.9377	26759	0.0487	7509	0.0137
10166	40598	0.9105	3383	0.0759	610	0.0137
10167	643455	0.9755	10015	0.0152	6162	0.0093
10168	30986	0.9247	2138	0.0638	384	0.0115
10169	331810	0.8871	37686	0.1008	4537	0.0121
10170	566268	0.9685	13877	0.0237	4530	0.0077
10171	174277	0.9233	12408	0.0657	2075	0.0110
10172	97975	0.9242	5838	0.0551	2197	0.0207
10173	153832	0.9426	7368	0.0451	1998	0.0122
10174	309692	0.9402	13426	0.0408	6275	0.0191
10175	482077	0.9370	26085	0.0507	6339	0.0123

10176	90404	0.7361	31139	0.2535	1273	0.0104
10177	395158	0.9086	35277	0.0811	4471	0.0103
10179	311827	0.9257	19402	0.0576	5637	0.0167
10180	287203	0.9325	20781	0.0675	24	0.0001
10181	20749	0.9043	2193	0.0956	2	0.0001
10182	348894	0.9494	15017	0.0409	3582	0.0097
10183	29678	0.9512	1088	0.0349	435	0.0139
10184	111615	0.8957	10048	0.0806	2954	0.0237
10185	401576	0.9272	23980	0.0554	7557	0.0174
10186	32950	0.9437	1760	0.0504	204	0.0058
10187	105428	0.8689	13163	0.1085	2746	0.0226
10188	261796	0.9383	11217	0.0402	6003	0.0215
10189	6127	0.9081	563	0.0834	57	0.0084
10190	442792	0.9108	34272	0.0705	9067	0.0187
10191	26814	0.9225	2105	0.0724	147	0.0051
10192	438044	0.9162	31570	0.0660	8483	0.0177
10193	165928	0.9413	9213	0.0523	1143	0.0065
10194	4267	0.9678	123	0.0279	19	0.0043
10195	59102	0.8876	5232	0.0786	2254	0.0338
10196	401846	0.9250	29206	0.0672	3357	0.0077
10197	30014	0.9180	2289	0.0700	391	0.0120
10198	415833	0.9446	21179	0.0481	3200	0.0073

The fraction read abundance was calculated using one of the following formulae:

reads for 500 cell control / [sum of reads from the 500, 100 and 20 cell controls]
reads for 100 cell control / [sum of reads from 500, 100 and 20 cell controls]
reads for 20 cell control / [sum of reads from 500, 100 and 20 cell controls]

The fraction read abundance allows for normalization of the read abundance between experimental samples that have varying read depth from MPS, and/or varying clone numbers and sizes. The fraction read abundance is then used to derive a correlation with known input cell number to calculate the approximate size of experimentally detected clones in absolute cell number (**Supplementary Figure 2A**).

Supplementary Table 1C **Fraction read abundance for secondary transplant data (22 samples)**

Library	500 cell control		100 cell control		20 cell control	
	Number of reads	Fraction read abundance	Number of reads	Fraction read abundance	Number of reads	Fraction read abundance
A23777	268877	0.9337	10010	0.0348	9067	0.0315
A23779	208913	0.9186	9565	0.0421	8943	0.0393
A23780	214487	0.9332	8576	0.0373	6770	0.0295
A23781	249936	0.9017	14024	0.0506	13220	0.0477
A23782	193583	0.9196	11838	0.0562	5093	0.0242
A23783	72082	0.9123	3612	0.0457	3313	0.0419
A23785	184038	0.9185	9321	0.0465	7005	0.0350
A23786	214828	0.9115	10432	0.0443	10416	0.0442
A23789	129747	0.9577	4575	0.0338	1153	0.0085
A23791	103798	0.9612	3716	0.0344	472	0.0044
A23794	4007	0.9375	175	0.0409	92	0.0215
A23795	140546	0.9248	6916	0.0455	4508	0.0297
A23796	151864	0.9049	8115	0.0484	7854	0.0468
A23797	221054	0.9166	15281	0.0634	4838	0.0201
A23800	224331	0.9547	7525	0.0320	3128	0.0133
A23806	80061	0.9255	4466	0.0516	1983	0.0229
A23807	242209	0.9262	9839	0.0376	9465	0.0362
A23808	118479	0.9423	5443	0.0433	1813	0.0144
A23811	140082	0.9269	6361	0.0421	4685	0.0310
A23813	160380	0.9315	6136	0.0356	5657	0.0329
A23815	79085	0.9195	3805	0.0442	3122	0.0363
A23819	84842	0.9233	4277	0.0465	2767	0.0301

Fraction read abundance is calculated and used similarly to the primary mice described above (**Supplementary Table 1B**).

Supplementary Table 2A Number of human hematopoietic cells collected from FACS from primary transplanted mice

		# FACS sorted cells in primary transplant					
		Mouse 1	Mouse 2			Mouse 1	Mouse 2
Week 4	Myeloid	34394	4054	Week 27 (Left leg)	Myeloid	1687	3447
	Erythroid	2941	0		Erythroid	109	36
	B cells	30317	2135		B cells	1528	489
	T cells	0	0		T cells	306	29396
	Total	67652	6189		Total	3630	33368
Week 9	Myeloid	1277	17934	Week 27 (Right leg)	Myeloid	2789	431
	Erythroid	0	64		Erythroid	418	0
	B cells	36956	1130000		B cells	2804	348
	T cells	0	0		T cells	456	44270
	Total	38233	1147998		Total	6467	45049
Week 16	Myeloid	4081	1990	Week 27 (Pelvis)	Myeloid	4256	290
	Erythroid	78	0		Erythroid	736	260
	B cells	10319	6614		B cells	5249	180
	T cells	160	451		T cells	377	3929
	Total	14638	9055		Total	10618	4659

Myeloid: CD45$^+$GFP$^+$CD15/33$^+$;
Erythroid: GFP$^+$human glyophorin A$^+$,
B cell: CD45$^+$GFP$^+$CD34$^-$CD19$^+$;
T cell: CD45$^+$GFP$^+$CD56$^-$CD3$^+$

Supplementary Table 2B Number of human hematopoietic cells collected from FACS from secondary transplanted mice

		# FACS sorted cells in secondary transplant					
		Mouse 1	Mouse 2			Mouse 1	Mouse 2
Week 4	Myeloid	1161	40	Week 16	Myeloid	393	60
	Erythroid	0	0		Erythroid	0	0
	B cells	0	0		B cells	1588	15
	T cells	0	0		T cells	0	0
	Total	1161	40		Total	1981	75
Week 8	Myeloid	40	17	Week 20	Myeloid	3	36
	Erythroid	0	0		Erythroid	0	13
	B cells	249	199		B cells	23	80
	T cells	0	0		T cells	0	0
	Total	289	216		Total	26	129
Week 12	Myeloid	30	27	Week 24*	Myeloid	73	0
	Erythroid	10	3		Erythroid	0	0
	B cells	266	25		B cells	87	17
	T cells	0	0		T cells	0	0
	Total	306	55		Total	160	17

Myeloid: CD45^{+}GFP^{+}CD15/33^{+};
Erythroid: GFP^{+}human glyophorin A^{+},
B cell: CD45^{+}GFP^{+}CD34^{-}CD19^{+};
T cell: CD45^{+}GFP^{+}CD56^{-}CD3^{+}
*: Bone marrow were collected and pooled from all sites (pelvis, left and right legs) at week 24 for analysis.

Supplementary Table 3A Sensitivity measurements for primary and secondary transplant samples

Transplant samples	Control	False negative clones	True positive clones	Sensitivity
Primary	500 cells	0/6	6/6	100
Primary	100 cells	0/6	6/6	100
Primary	20 cells	2/6	4/6	67
Secondary	500 cells	0/11	11/11	100
Secondary	100 cells	5/11	6/11	55
Secondary	20 cells	5/11	6/11	55

Supplementary Table 3B **Specificity measurements for primary and secondary transplant samples**

Transplant samples	Control	False positive clones	True negative clones	Specificity
Primary	1	0/3	134	100.00
Primary	2	0/3	177	100.00
Primary	3	0/3	87	100.00
Primary	4	1/3	128	99.22
Primary	5	0/3	177	100.00
Primary	6	0/3	260	100.00
Primary	Average			99.87
Secondary	1	0	56	100
Secondary	2	0	181	100
Secondary	3	0	169	100
Secondary	4	0	111	100
Secondary	5	0	91	100
Secondary	6	0	136	100
Secondary	7	0	118	100
Secondary	8	0	98	100
Secondary	9	0	55	100
Secondary	10	0	234	100
Secondary	11	0	125	100
Secondary	Average			100

Supplementary Table 5 **2 x 2 contingency table of T-cell producing activity of all week 27 clones at end-point of primary transplant versus secondary repopulating activity**

Number of clones at 27 weeks post-transplant in primary mice with:			
	detectable T-cell production	no detectable T-cell production	Total
Secondary repopulation	3	3	6
No secondary repopulation	6	19	25
Total	9	22	31

P-value of Fisher's exact test = 0.32

Supplementary Table 6 Mature cells (x 10^3) in clones detected only in secondary recipients

		Clone S2			Clone S3			Clone S11			Clone S1			Clone S4		
	Week	M	B	T	M	B	T	M	B	T	M	B	T	M	B	T
Secondary recipients	4	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	8	-/37	-/-	-/-	22/-	-/-	-/-	-/-	-/-	-/-	-/-	-/0.7	-/-	-/-	-/-	-/-
	12	-/-	-/-	-/-	-/-	-/46	-/-	-/-	76/6	-/-	-/-	-/-	-/-	-/-	-/41	-/-
	16	-/-	33/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	20	-/-	-/4	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	24	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-

		Clone S5			Clone S6			Clone S7			Clone S8			Clone S9		
	Week	M	B	T	M	B	T	M	B	T	M	B	T	M	B	T
Secondary recipients	4	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	8	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	3/-	-/-
	12	-/-	-/38	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	16	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	20	-/-	-/-	-/-	9/-	-/-	-/-	-/45*	-/-	-/-	-/4*	-/-	-/-	-/-	-/-	-/-
	24	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-

		Clone S10			Clone S12			Clone S13			Clone S14			Clone S15		
	Week	M	B	T	M	B	T	M	B	T	M	B	T	M	B	T
Secondary recipients	4	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	8	-/-	1/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	12	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	16	-/-	-/-	-/-	14/-	-/-	-/-	5/-	-/-	-/-	-/-	18/-	-/-	-/-	-/-	-/-
	20	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	24	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	5/-	-/-	-/-

		Clone S16			Clone S17			Clone S18			Clone S19			Clone S20		
	Week	M	B	T	M	B	T	M	B	T	M	B	T	M	B	T
Secondary recipients	4	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	8	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	12	-/-	-/-	-/-	-/-	-/-	-/-	-/9	-/-	-/-	-/8	-/-	-/-	-/7	-/-	-/-
	16	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	20	-/-	-/-	-/-	-/-	41/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
	24	3/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-

		Clone S21			Clone S22		
	Week	M	B	T	M	B	T
Secondary recipients	4	-/-	-/-	-/-	-/-	-/-	-/-
	8	-/-	-/-	-/-	-/-	-/-	-/-
	12	-/2	-/-	-/-	-/-	-/6	-/-
	16	-/-	-/-	-/-	-/-	-/-	-/-
	20	-/-	-/-	-/-	-/-	-/-	-/-
	24	-/-	-/-	-/-	-/-	-/-	-/-

*Only erythroid output recorded. M: myeloid; B: B cells; T: T cells; -: no detectable output

Supplementary Table 7 “New” clones detected in secondary recipients (but below the threshold of detection in the primary recipient)

"New" Secondary clone	Barcode sequence	Sample detected	# Reads
S1	GCATCTTGATCGAAAGCGGTCCAACTC	27 week (right femur): T-cells	148
		27 week (left femur): T-cells,	2
S3	TGATCCAGATCGAAACTGGTCTAACCG	16 week (femur): Myeloid cells	42
		27 week (right femur): B-cells,	2
S5	CAATCCCGATCCAAACCGGTCCAACCT	27 week (right femur): T-cells,	65
S7	CCATCAGGATGGAAAAGGGTGGAACGG	4 week (femur): Myeloid cells	14
S11	TGATCGAGATCGAAATGGGTGCAACGG	27 week (right femur): T-cells,	188
		27 week (pelvis): Myeloid cells,	78

SUPPLEMENTARY FIGURE LEGENDS

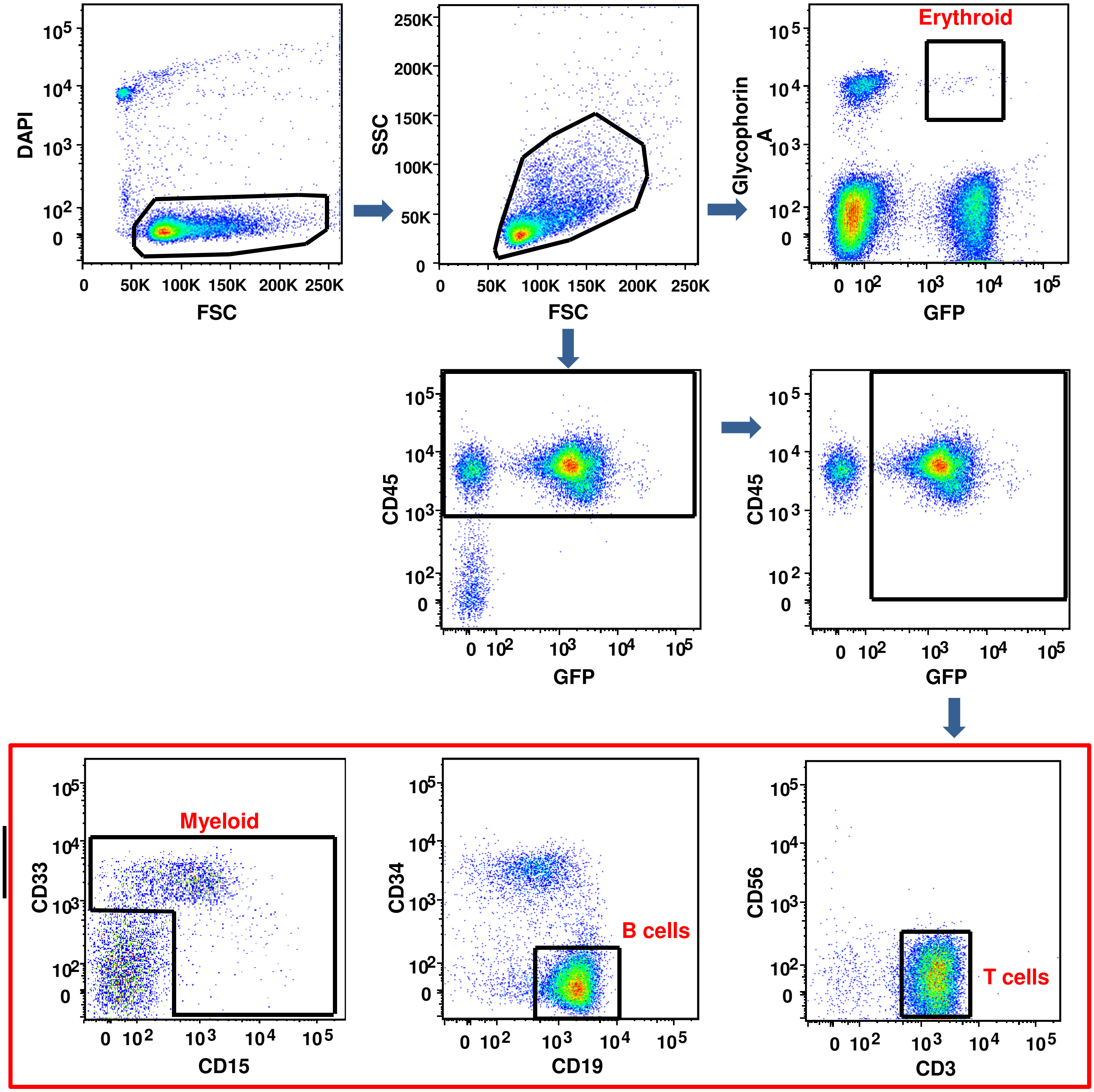
Supplementary Fig. 1 FACS sorting of various lineages of human hematopoietic cells from mouse bone marrow. Representative FACS plots showing the sorting strategy used for the collection of various human cell lineages from mouse BM samples. Erythroid=GFP$^+$human glyophorin A$^+$, Myeloid=CD45$^+$GFP$^+$CD15/33$^+$; B cell=CD45$^+$GFP$^+$CD34$^-$CD19$^+$; T cell=CD45$^+$GFP$^+$CD56$^-$CD3$^+$.

Supplementary Fig. 2 Determination of cell numbers from barcode read abundance. **(A)** Polynomial regressions for relationships determined for analysis of primary (56 control samples) and secondary (22 control samples) transplant samples. Shaded regions in gray represent 95% confidence intervals. **(B)** Correlation of cell numbers identified by FACS and MPS analysis. Each data point represents an individual cell sample sorted by FACS and also analyzed by MPS.

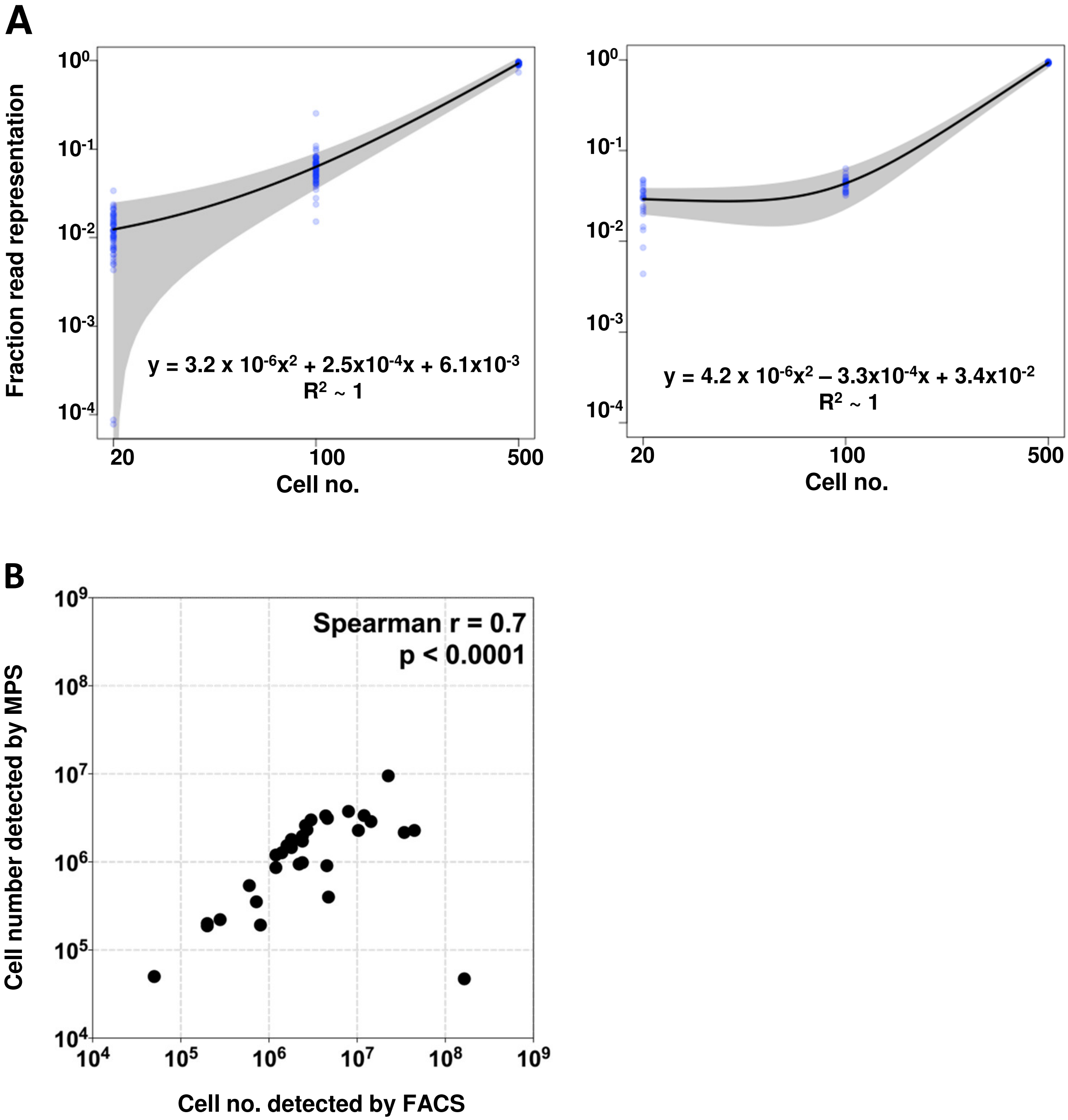
Supplementary Fig. 3 Lineage outputs of all clones detected. **(A)** Heatmap of the lymphoid (red) and myeloid (blue) cell contribution of all 196 clones tracked over time in primary and secondary mice. The contribution to each clone is represented by a horizontal bar of increasing intensity according to the number of cells it contained of that lineage type so that the plot resembles a virtual Southern blot. **(B)** Lineage outputs by clones that appeared in primary mice and persisted until 27 weeks post-transplant. Number in each plot indicates the respective clone ID.

Supplementary Fig. 4 **(A)** Percentage of week 27 clones detectable at the indicated BM sites in each of the primary transplanted mouse. The total number of clones detected in each mouse is also indicated. (**B**) GM/(B+T) ratio of all 16 clones at different BM sites at week 27 in the 2 primary mice. (**C**) Clones having a size of >5.5 x 10^5 cells display a smaller standard deviation (SD) of the GM/(B+T) ratios determined at different BM sites (left panel). Change in GM/(B+T) ratio both within clones and in the differentiation features of clones that appeared at successively later times are shown (middle and right panel). Serial analysis of the relative lineage output of 8 of the 27 week clones that display ≥10-fold increase (middle panel) compared to only 1 clone with ≥10-fold decrease (right panel) in their GM/(B+T) ratio within the 27 weeks post-transplant.

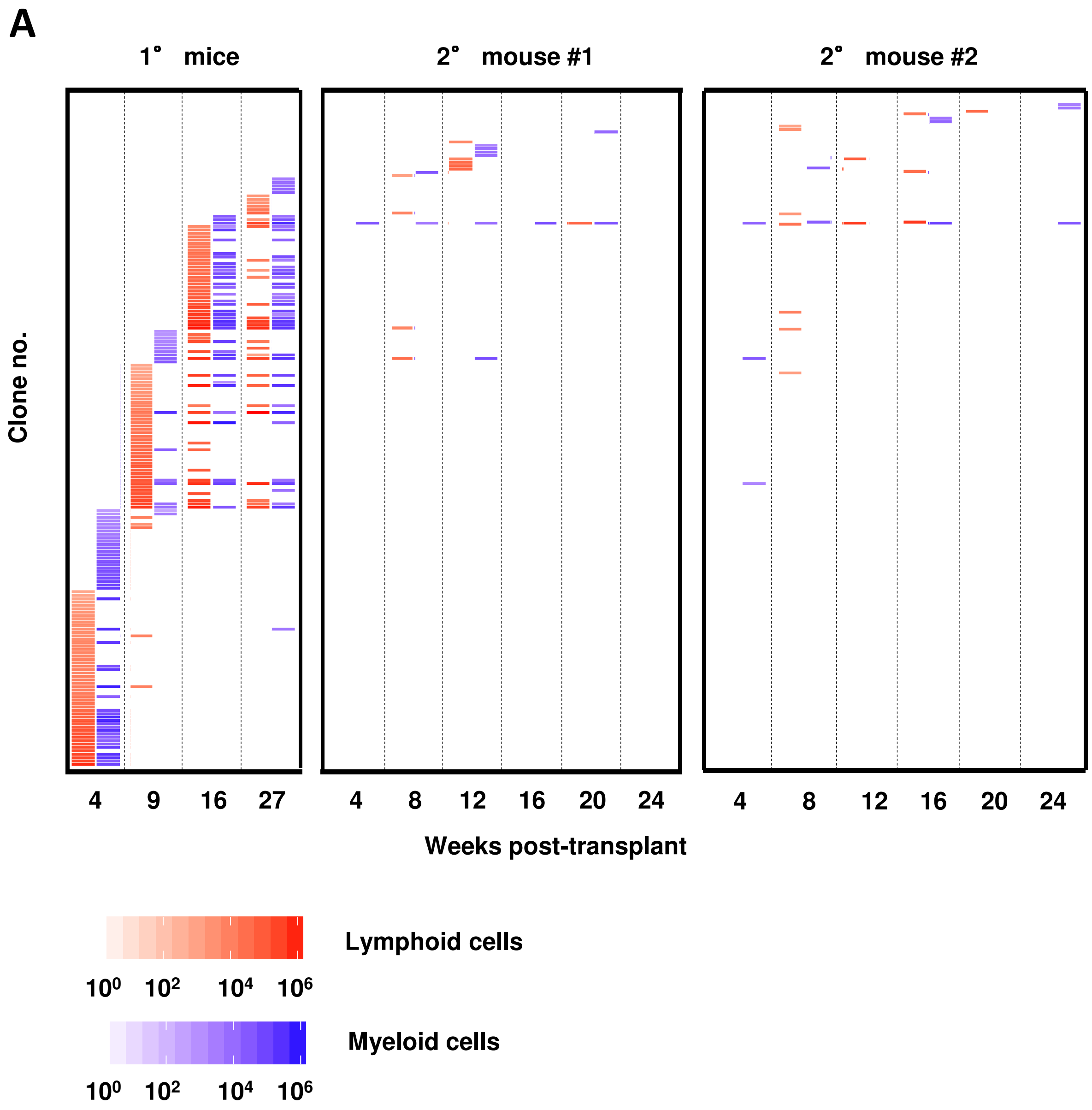
Supplementary Figure 1



Supplementary Figure 2

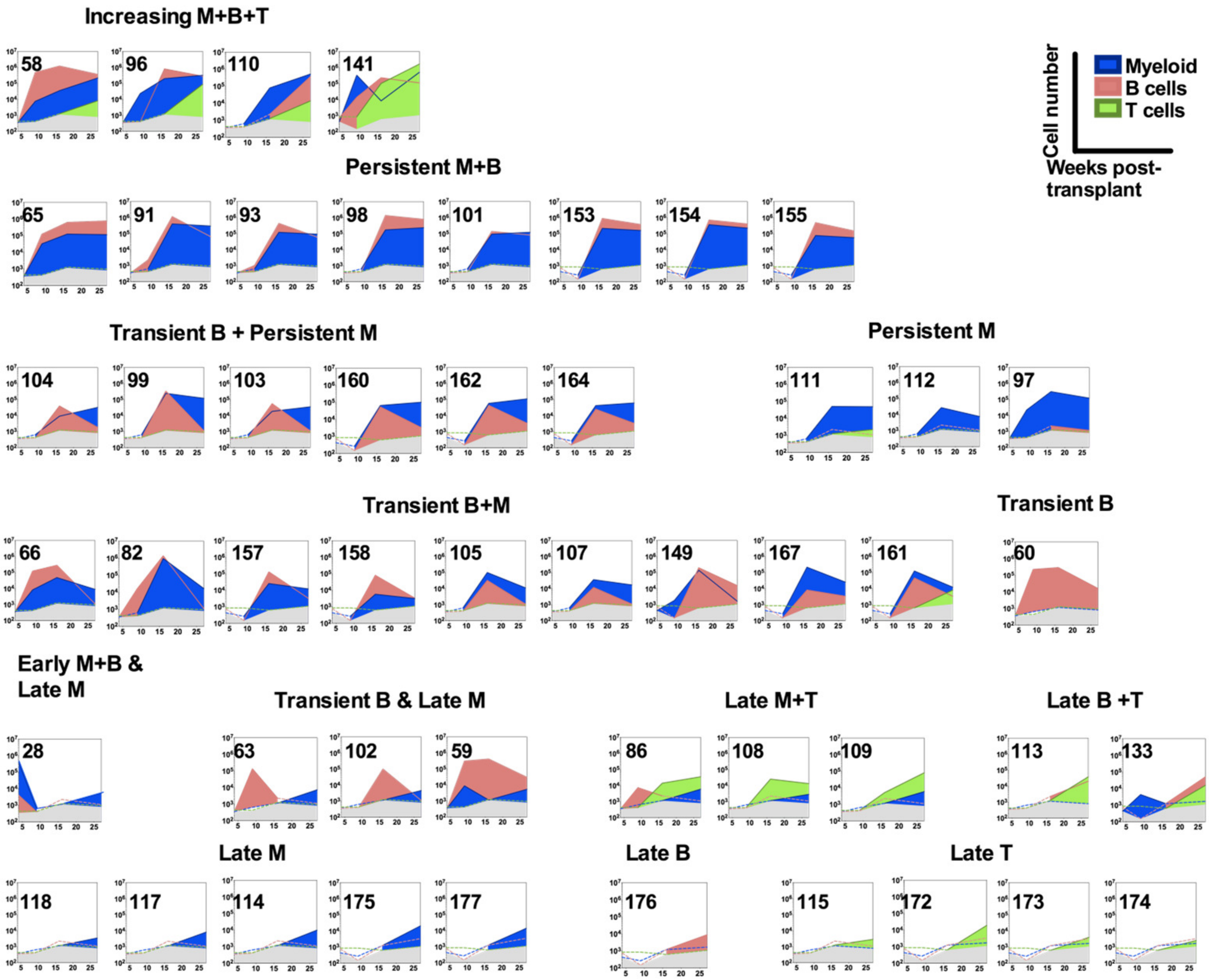


Supplementary Figure 3

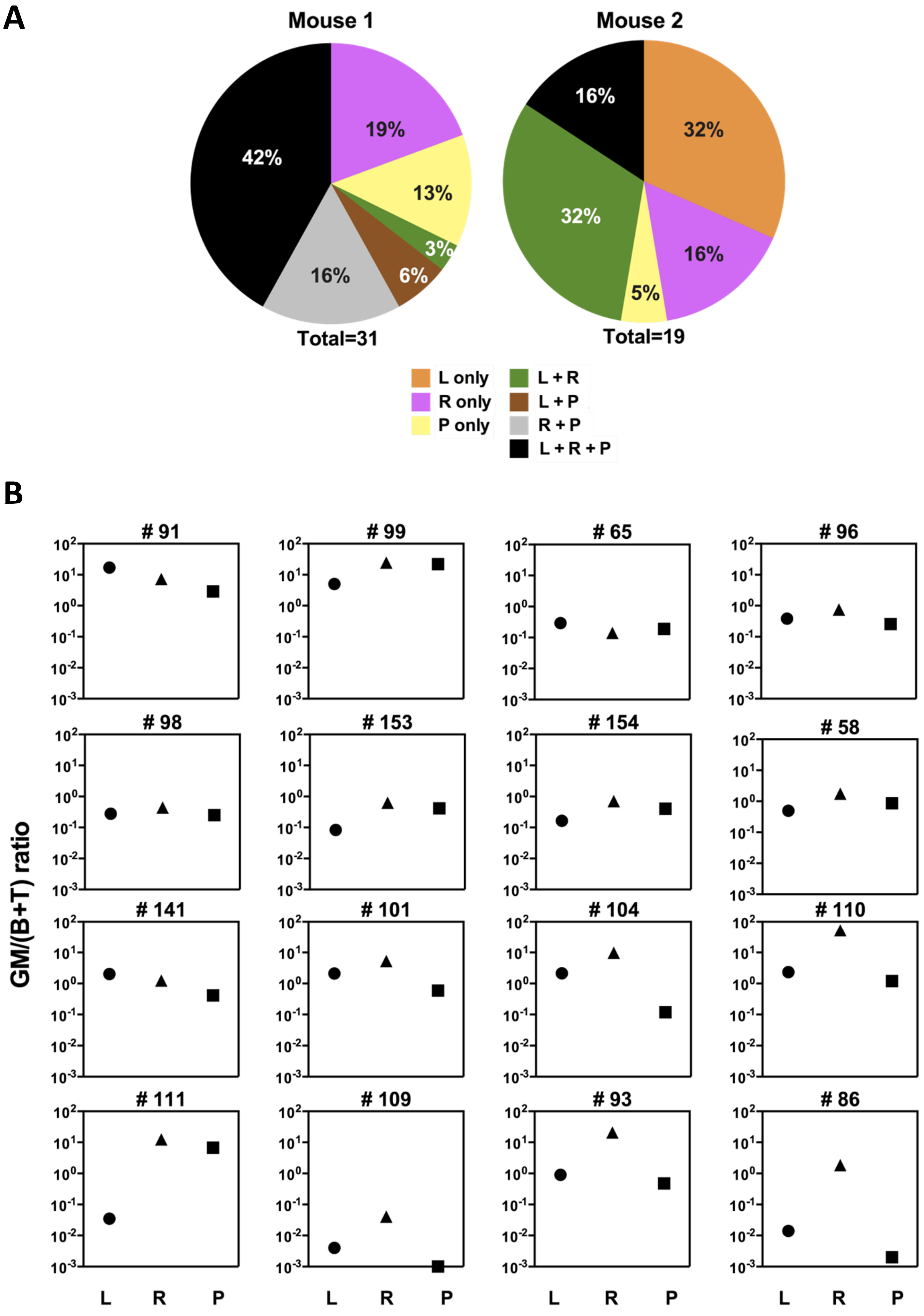


Supplementary Figure 3

B



Supplementary Figure 4



Supplementary Figure 4

C