Supplementary Table 1:

Number of TCRβ sequence reads for each patient at each time point.

Participant ID	*CD4-Baseline*	*CD8-Baseline*	*CD4-Month 2*	*CD8-Month 2*	*CD4-Month 12*	*CD8-Month 12*
P1	5,911,743	3,307,026	6,673,545	1,239,676	71,735	3,521,259
P2	7,492,562	9,089,827	3,536,592	2,489,318	3,623,401	3,137,842
P3	10,615,706	4,676,193	4,941,424	1,549,568	1,711,014	2,729,958
P4	7,357,408	6,465,900	3,491,905	2,108,929	3,116,450	4,201,397
P5	13,845,887	5,264,852	4,702,715	1,391,932	3,698,921	3,171,303
P6	12,843,195	3,397,798	7,083,610	884,345	5,701,818	3,275,940
P7	8,233,572	4,183,501	3,525,754	1,117,136	4,889,139	3,502,104
P8	5,673,255	6,862,865	2,389,466	1,566,288	7,167,425	2,629,030
P9	8,663,334	6,173,276	6,332,162	3,031,036	6,425,982	7,537,941
P10	10,734,048	4,072,091	6,957,898	2,286,055	7,510,644	5,145,083
P11	6,841,239	2,175,496	2,438,224	1,762,509	7,152,565	2,403,616
P12	7,303,883	3,211,597	2,028,536	852,193	4,656,752	4,118,116
P13	15,578,596	5,992,025				
P14	8,364,553	5,080,137			5,295,304	5,338,283
P15	14,211,595	5,801,929	6,734,724	2,786,423	3,334,278	3,087,199
P16	10,019,067	5,624,137	2,643,392	1,714,703	2,788,528	2,642,254
P17	4,456,577	3,249,466	1,511,062	1,128,754	2,427,342	5,823,929
P18	5,207,443	3,586,040	1,777,994	2,290,060	3,028,695	3,340,288
P19	10,611,046	7,581,629	3,135,784	3,228,351	7,625,755	3,005,181
P20	6,277,461	4,700,292	5,268,726	2,253,546	4,609,747	4,548,108
P21	9,114,808	8,106,962	2,433,443	788,463	3,977,142	3,487,372
P22	10,389,460	5,431,145	4,276,661	1,357,960		
P23	9,632,294	5,763,717	5,555,145	1,352,120	7,229,813	4,912,558
P24	3,197,894	9,528,194	9,871,778	1,005,294	3,950,830	5,195,022
P25	5,724,422	1,503,661	1,974,599	2,962,907	4,010,050	6,258,656

Supplementary Table 2:

Frequency of known EBV-specific public clones restricted by HLA:A2 or B8 in CD4+ or CD8+ blood from patients at baseline and 2 months post-transplant. Data, including EBV status, is shown for all patients, grouped by whether they are A2+, B8+ or neither.

A: HLA:A2

	Patient #			CASSLGQAYEQYF	CASSSGQAYEQYF	CSVGSGGTNEKLFF	CSVGTGGTNEKLFF	Time post-Tx EBV infection confirmed
EBV reactivation	P21	CD4+	Baseline	2.28E-06	3.00E-06	6.00E-06	9.80E-05	31 days
			2 months	0	0	2.90E-02	4.02E-02	
		CD8+	Baseline	4.16E-05	4.27E-06	9.51E-04	7.15E-03	
			2 months	0	0	9.16E-02	2.54E-01	
New EBV infection	P1	CD4+	Baseline	2.28E-06	1.60E-05	0	1.88E-06	218 days
			2 months	0	0	0	2.00E-06	
		CD8+	Baseline	3.91E-07	0	0	4.77E-03	
			2 months	0	0	0	0	
	P3	CD4+	Baseline	0	0	0	0	50, 56, 71 days
			2 months	1.57E-06	0	3.85E-04	7.96E-04	
		CD8+	Baseline	1.38E-05	0	9.99E-06	8.56E-06	
			2 months	0	0	2.68E-03	1.14E-02	
	P14	CD4+	Baseline	4.92E-05	2.83E-05	0	0	43 days
			2 months	no data				
		CD8+	Baseline	2.46E-05	0	4.37E-06	9.90E-04	
			2 months	no data				
EBV infected, no reactivation	P4	CD4+	Baseline	2.84E-07	2.13E-06	0	0	N/A
			2 months	0	0	0	0	
		CD8+	Baseline	2.41E-06	0	0	5.79E-04	
			2 months	0	0	0	0	
	P18	CD4+	Baseline	3.53E-06	0	2.60E-06	0	
			2 months	0	0	1.67E-03	3.99E-04	
		CD8+	Baseline	9.03E-06	6.94E-07	1.41E-03	2.57E-03	
			2 months	0	0	1.73E-01	2.55E-02	
	P19	CD4+	Baseline	2.86E-05	1.10E-05	0	0	
			2 months	0	0	9.19E-04	0	
		CD8+	Baseline	2.99E-05	1.15E-05	9.58E-05	2.38E-06	
			2 months	0	0	6.26E-02	4.44E-06	
	P22	CD4+	Baseline	2.57E-06	2.99E-06	0	0	
			2 months	0	0	0	0	
		CD8+	Baseline	2.83E-06	4.64E-06	0	4.85E-06	
			2 months	0	0	0	5.94E-04	
No EBV infection	P15	CD4+	Baseline	5.44E-06	1.25E-05	0	0	N/A
			2 months	0	0	0	1.33E-04	
		CD8+	Baseline	9.64E-06	0	3.06E-05	1.32E-03	
			2 months	0	0	4.42E-05	1.30E-02	

B: HLA:B8

	Patient #			CASSLGQAYEQYF	Time post-Tx EBV infection confirmed
EBV reactivation	P8	CD4+	Baseline	0	57, 167 days
			2 months	0	
		CD8+	Baseline	1.77E-05	
			2 months	0	
	P10	CD4+	Baseline	3.70E-05	74 days
			2 months	2.64E-04	
		CD8+	Baseline	3.15E-02	
			2 months	4.64E-03	
New EBV infection	P23	CD4+	Baseline	5.84E-06	75 days
			2 months	6.84E-04	
		CD8+	Baseline	4.53E-03	
			2 months	2.78E-02	
EBV infected, no reactivation	P12	CD4+	Baseline	3.21E-05	N/A
			2 months	1.72E-03	
		CD8+	Baseline	3.39E-04	
			2 months	2.86E-02	
	P13	CD4+	Baseline	8.69E-06	
			2 months	no data	
		CD8+	Baseline	3.53E-05	
			2 months	no data	
	P20	CD4+	Baseline	1.42E-06	
			2 months	0	
		CD8+	Baseline	5.18E-06	
			2 months	0	
No EBV infection	P2	CD4+	Baseline	4.78E-06	N/A
			2 months	9.01E-06	
		CD8+	Baseline	4.97E-07	
			2 months	0	

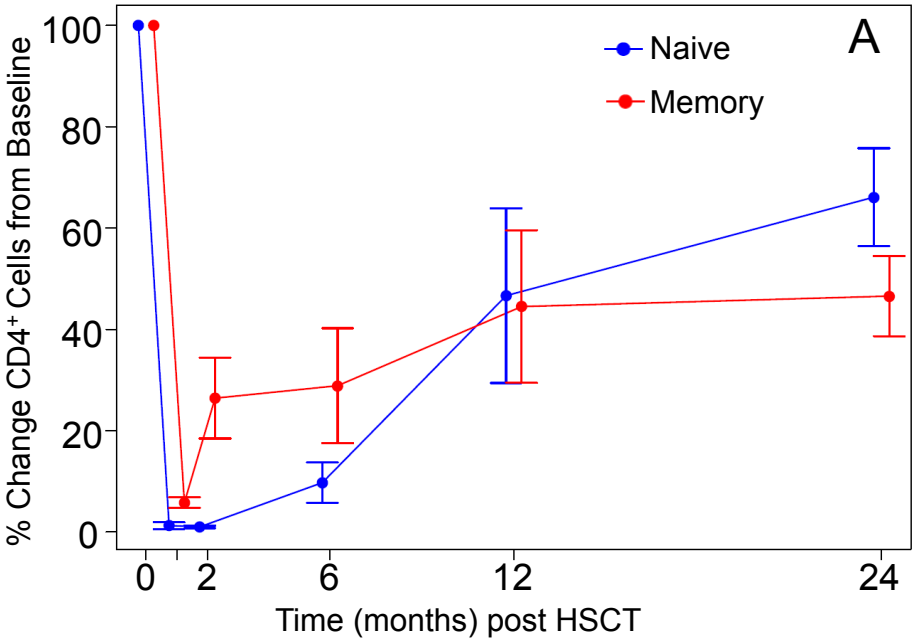
C: - non HLA :A2, non HLA:B8

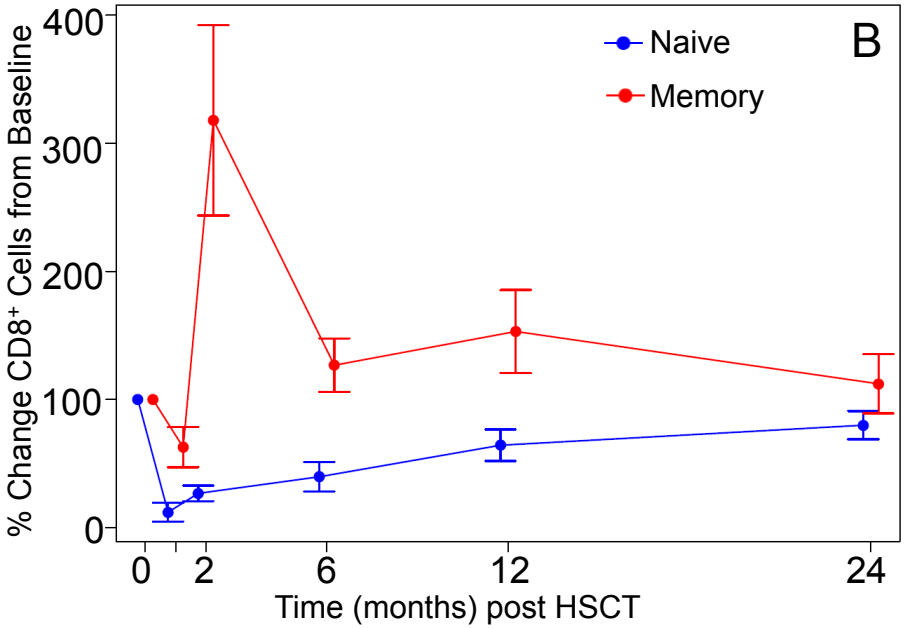
	Patient #			CASSLGQAYEQYF	CASSSGQAYEQYF	CSVGSGGTNEKLFF	CSVGTGGTNEKLFF	Time post-Tx EBV infection confirmed
EBV reactivation	P6	CD4+	Baseline	1.69E-06	4.51E-07	0	0	26 days
			2 months	0	0	5.34E-06	4.65E-06	
		CD8+	Baseline	1.65E-05	0	0	0	
			2 months	0	0	0	1.69E-05	
	P11	CD4+	Baseline	0	0	0	0	72 days
			2 months	0	0	4.32E-04	3.89E-04	
		CD8+	Baseline	0	7.39E-06	0	0	
			2 months	0	0	0	0	
	P16	CD4+	Baseline	4.98E-06	2.49E-06	0	0	26,28,33,35,36,40,42,47, 53,62,67,81,102 days
			2 months	0	0	0	0	
		CD8+	Baseline	2.63E-06	0	0	0	
			2 months	0	0	0	3.60E-05	
	P24	CD4+	Baseline	6.93E-05	3.33E-06	0	0	36 days
			2 months	0	0	0	1.18E-06	
		CD8+	Baseline	3.48E-05	4.23E-06	0	0	
			2 months	0	0	1.24E-05	0	
New EBV infection	P17	CD4+	Baseline	1.75E-05	0	0	0	13 days
			2 months	0	0	0	0	
		CD8+	Baseline	1.39E-05	0	0	0	
			2 months	0	0	0	0	
EBV infected, no reactivation	P5	CD4+	Baseline	1.54E-07	0	0	0	N/A
			2 months	0	0	0	0	
		CD8+	Baseline	1.26E-05	0	0	0	
			2 months	0	0	9.54E-06	2.33E-05	
	P7	CD4+	Baseline	0	0	0	0	
			2 months	0	0	0	0	
		CD8+	Baseline	0	0	0	0	
			2 months	4.35E-06	0	0	7.83E-06	
	P9	CD4+	Baseline	0	7.12E-07	0	0	
			2 months	1.14E-05	0	0	0	
		CD8+	Baseline	7.10E-06	0	0	0	
			2 months	0	0	4.00E-06	0	
No EBV infection	P25	CD4+	Baseline	0	0	0	0	N/A
			2 months	0	0	0	0	
		CD8+	Baseline	1.38E-06	0	0	0	
			2 months	0	0	0	0	

Supplementary Figures

T Cell Repertoire Following Autologous Stem-Cell Transplantation for Multiple Sclerosis

Paolo A. Muraro, Harlan Robins, Sachin Malhotra, Michael Howell, Deborah Phippard, Cindy Desmarais, Alessandra de Paula Alves Sousa, Linda M. Griffith, Noha Lim, Richard A. Nash, and Laurence A. Turka

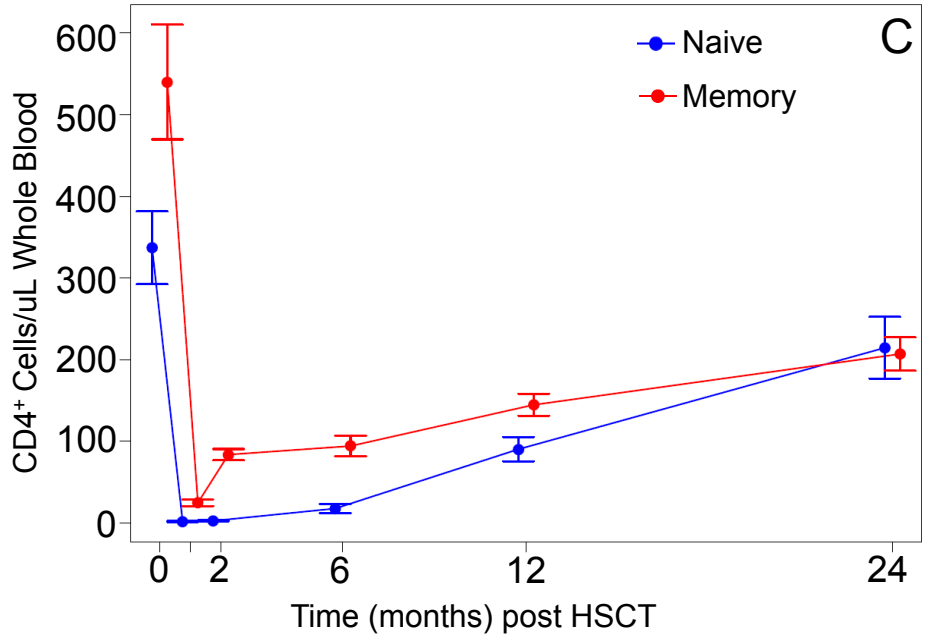


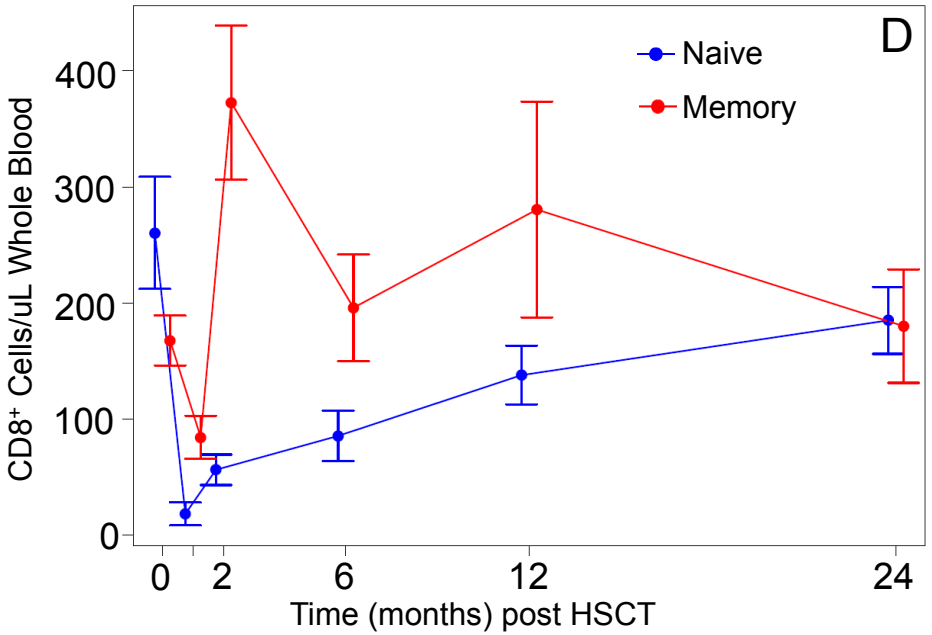


Supplementary Figure 1

Kinetics of immune reconstitution following HSCT.

$CD4^+$ cells (Panel A) and $CD8^+$ Cells (Panel B) for the 25 participants treated with HSCT and their reconstitution kinetics post autologous CD34+ hematopoietic stem cell transplant. Data are depicted as mean ± standard error normalized to a baseline of 100%. Naïve cells ($CD45RA^+$) were more effectively depleted following HSCT than memory cells ($CD45RO^+$), with $CD4^+$ and $CD8^+$ naïve cells at 1.3% (1.9 cells/uL) and 12.1% (18.4 cells/uL) of baseline, respectively, 1 month post-HSCT, and $CD4^+$ and $CD8^+$ memory cells constituting 5.4% (24.8 cells/uL) and 71.2% (84.4 cells/uL) of their baseline levels. Time points for collection were 0, 1, 2, 6, 12, and 24 months. The horizontal axis is eccentric to avoid data point overlap in the graph.



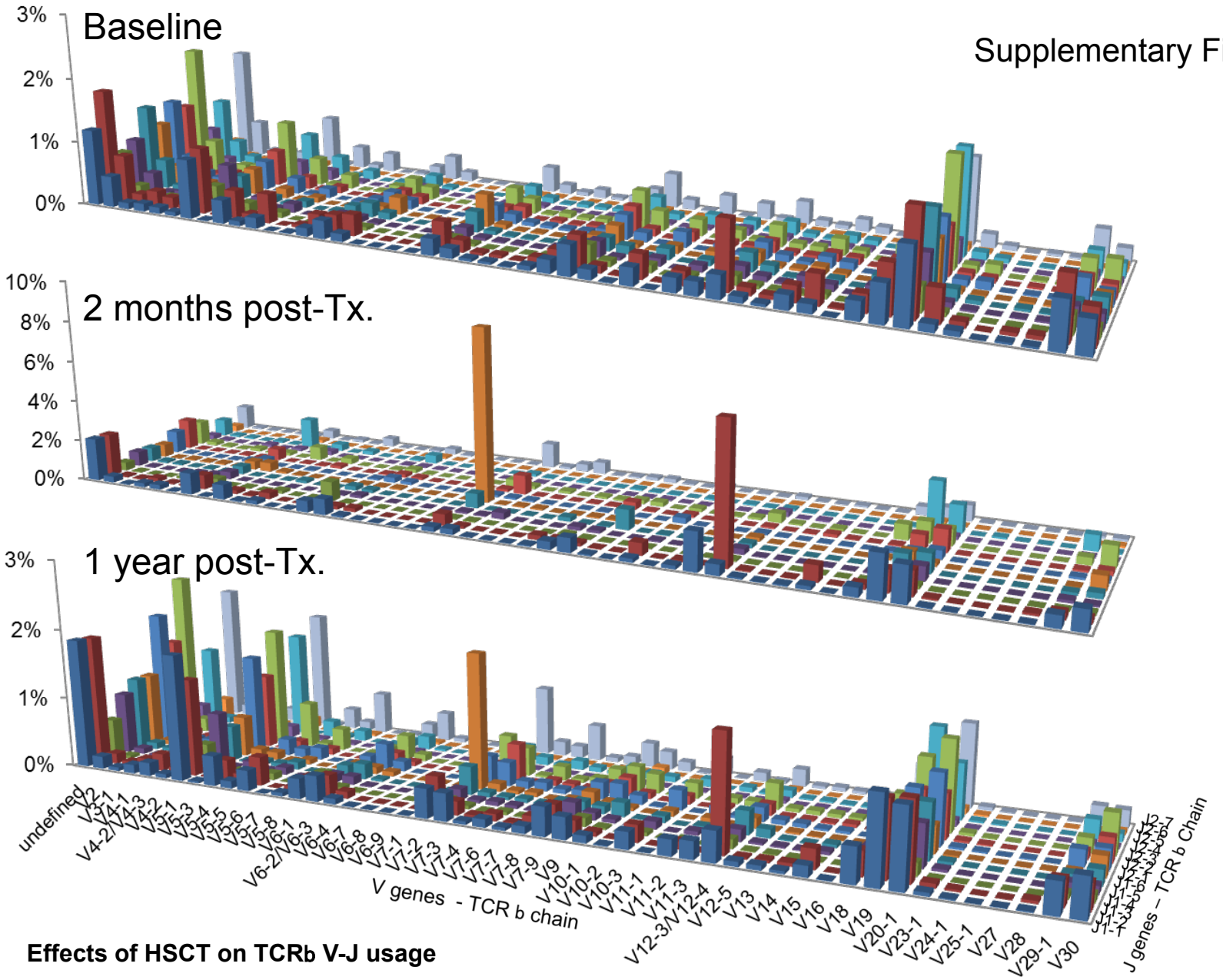


Supplementary Figure 1

Kinetics of immune reconstitution following HSCT.

$CD4^+$ cells (Panel C) and $CD8^+$ Cells (Panel D) for the 25 participants treated with HSCT and their reconstitution kinetics post autologous CD34+ hematopoietic stem cell transplant. Data are depicted as the mean of the absolute counts from whole blood ± standard error. Time points for collection were 0, 1, 2, 6, 12, and 24 months. The horizontal axis is eccentric to avoid data point overlap in the graph.

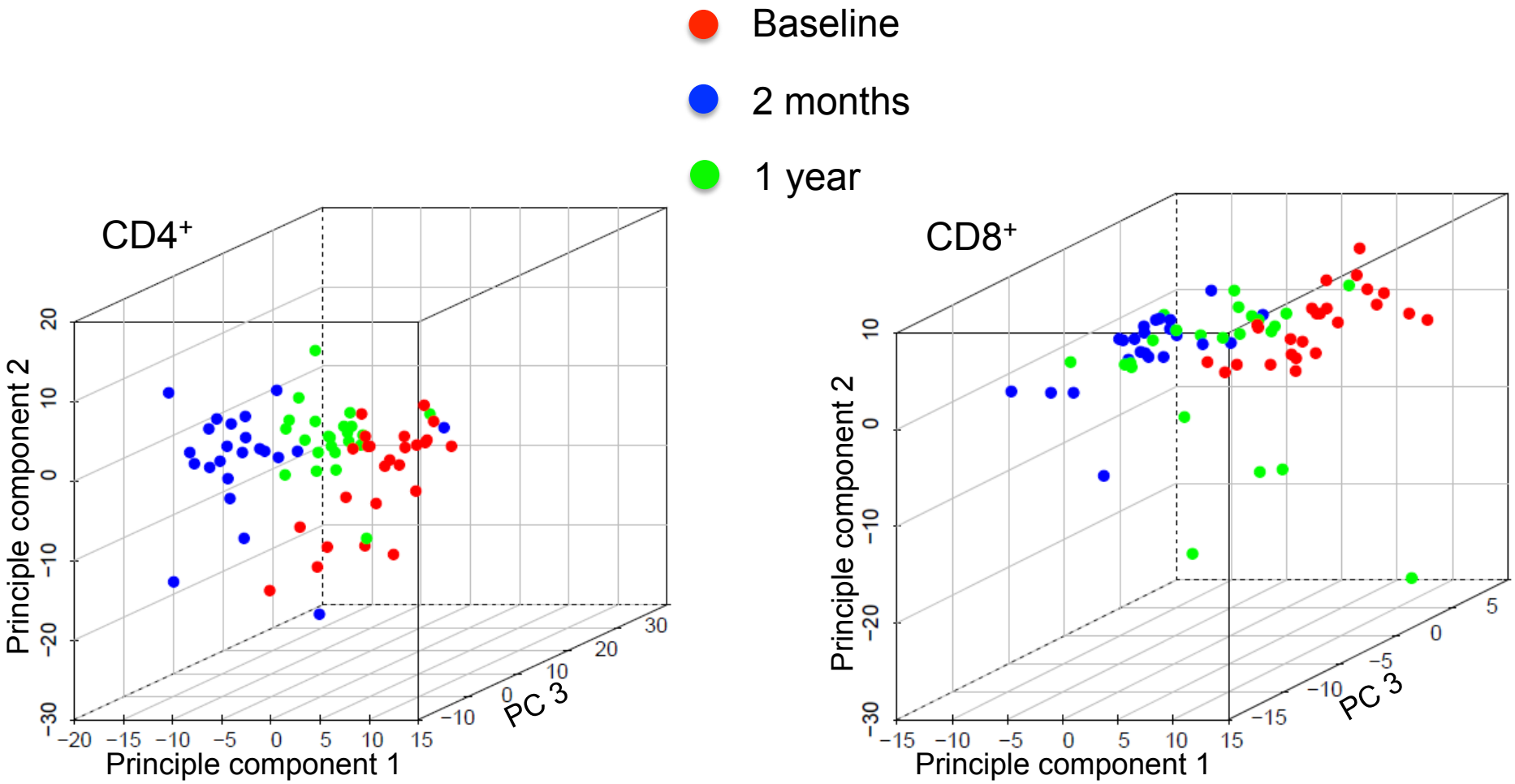
Supplementary Figure 2A



Effects of HSCT on TCRb V-J usage

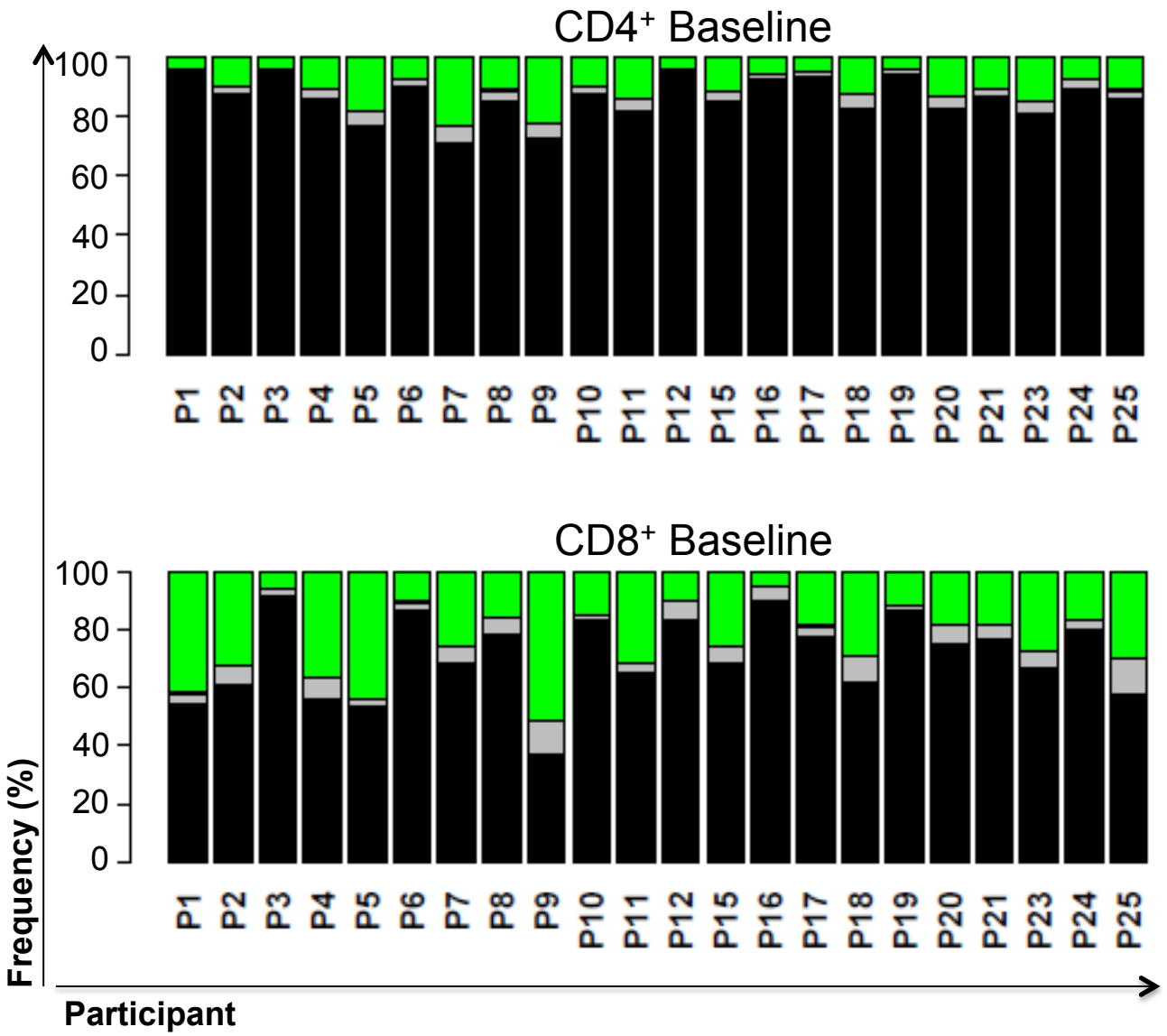
Representation of frequency of V-J utilization in TCRb sequences for CD4 T cells from one trial participant chosen at random at 2 months (top) and 12 months (bottom) post-HSCT. Note different y-axis scale for the two time points.

Supplementary Figure 2B



Multidimensional scaling analysis of V-J recombination frequencies in CD4+ (left) and CD8+ (right) cells across multiple individuals and time points.

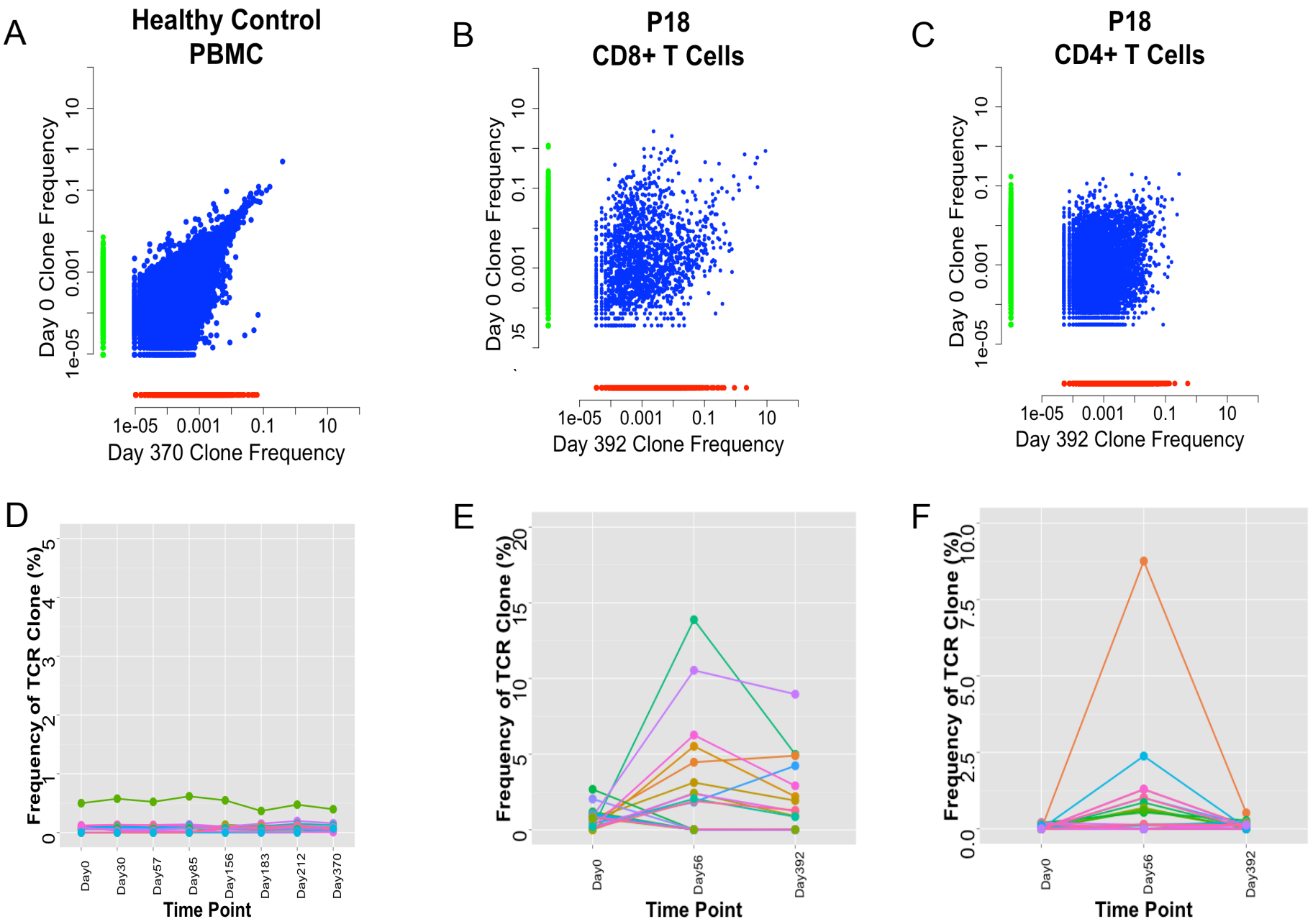
Supplementary Figure 3



Effect of HSCT on baseline TCR repertoire

Proportional representation of the "fate" of clones present before therapy. Clones are characterized as having been ablated (no longer detectable 12 months post-transplant), depleted (detectable, but reduced in frequency – see figure 2A and 2C) or persistent (detectable at the same frequency level).

Supplementary Figure 4



Supplementary Figure 4

TCR repertoire temporal dynamics for healthy control PBMCs versus transplant recipient CD8+ and CD4+ T cells over a one year period.

A) Scatterplot of TCR clone frequencies at Day 0 versus Day 370 in a healthy control subject. Blue points indicate shared clones, red points are TCRs found at Day 370 but absent from the Day 0 time point, green points are TCRs found at Day 0 but absent from Day 370. The repertoire is relatively stable over a one year period. B) TCR repertoire at Day 0 versus Day 392 for CD8+ T cells sorted from transplant recipient P18. Although many TCRs appear at both time points, a larger percentage of the repertoire at Day 392 is made up of clones that were absent from Day 0 (red points). As well, many clones prevalent at Day 0 are no longer seen at Day 392 (green points). C) TCR repertoire at Day 0 versus Day 392 for CD4+ T cells sorted from transplant recipient P18. D) Clone tracking of the 10 most frequent clones at each time point, measured over all available time points, in PBMCs from a healthy control subject. The most frequent clone is present at <1% at Day 0 and Day 370. Clone frequencies for the most expanded clones remain relatively stable over the course of one year. E) Clone tracking of the 10 most frequent clones for CD8+ T cells from transplant recipient P18. The top 10 clones represent a larger fraction of the overall repertoire at all time points; as well, the repertoire exhibits considerable fluctuation over a one year period, with novel clones rising to high frequency by Day 392. F) Clone tracking of the 10 most frequent clones for CD4+ T cells from transplant recipient P18. The repertoire exhibits considerable fluctuation between Day 0 and Day 56, and between Day 56 and Day 392.

Supplementary Information

T Cell Repertoire Following Autologous Stem-Cell Transplantation for Multiple Sclerosis

Paolo A. Muraro[1*], Harlan Robins[2*], Sachin Malhotra[3], Michael Howell[3], Deborah Phippard[3], Cindy Desmarais[2], Alessandra de Paula Alves Sousa[1], Linda M. Griffith[4], Noha Lim[3], Richard A. Nash[5], and Laurence A. Turka[3,6]

Supplementary Methods

Flow Cytometry. Whole blood was lysed and stained with fluorochrome labeled antibodies directed against CD3, CD4, CD8, CD45RO, and CD45RA (Becton Dickinson) and evaluated using a Canto A flow cytometer (Becton Dickinson) and populations identified through sequential manual gating in Flowjo (TreeStar Inc., Ashland, OR).

Cell Separation and DNA Isolation. PBMCs frozen in 20% DMSO/human AB serum were thawed and then separated by sequential positive selections for CD4+ cells (Miltenyi Biotec; Catalog # 130-045-101) followed by CD8+ cells (Miltenyi Biotec; Catalog # 130-045-201) per manufacturer's instructions. DNA was isolated using the Qiagen QIAmp DNA mini kit (Qiagen; Catalog # 51306) per manufacturer's instructions except the elution volume was increased to 100 µL for use in TCR sequencing.

Twenty-five patients had autologous hematopoietic stem cells collected for the clinical trial with an average age of 37 yrs, (range 27-53). There were 17 females and 8 males with an average disease duration of 6.4 yrs. (1.0-13.0). Their baseline EDSS (expanded disability status scale) ranged from 3.0 to 5.5 with a mean value of 4.5. All 25 subjects were mobilized and an autologous graft collected; 24 of these proceeded to transplantation.

Additional clinical details on the HALT-MS study. Subjects received a standard BEAM preparative regimen of BCNU (300 mg/m^2, day -6), etoposide (100 mg/m^2/day, BID days -5 to -2), ara-C (100 mg/m^2/day, BID days -5 to -2) and melphalan (140 mg/m^2, day -1) with rabbit antithymocyte globulin (ATG, 2.5 mg/kg/day on days -2 and -1). The graft, Auto-CD34+HPC, was manufactured under a Type II Drug Master File (FDA BB-IND-11821, V1.0 July 14, 2004). CD34+ cells were selected using the Baxter Isolex

device. The Certificate of Analysis specified CD34+ selected cells of $\geq$ 70% with other cells including T cells (CD3+), B cells (CD19+), NK cells (CD56+) and monocytes (CD14+) $\leq$ 30% combined.

On day 0, the autologous cell graft was thawed and infused. Patients received a minimum of 2.0 x 10^6 $CD34^+$ cells/kg of patient body weight per the protocol; the median $CD34^+$ dose was 4.58 (range 2.95 - 9.73) x 10^6 cells/kg, and the average CD3+ dose was 4 x 10^4 cells/kg. HALT-MS subjects engrafted neutrophils with an ANC 500 at a median of day 11, and platelets at 20,000/uL on two consecutive measurements on different days, with no platelet transfusions in the preceding seven days,at a median of day 18. Treatment failure (i.e., non-responder status) was defined as one of more of: death from any cause, disease activity/disability as evidenced by an increase of greater than >0.5 in EDSS confirmed at 3 months or later, presence of 2 or more independent MRI lesions indicative of disease activity, or clinical relapse/deterioration. HALT-MS subjects engrafted neutrophils with an ANC greater than 500 cells/uL at a median of day 11, and platelets at greater than 20,000/uL at a median of day 17.