Supplemental Figure 1
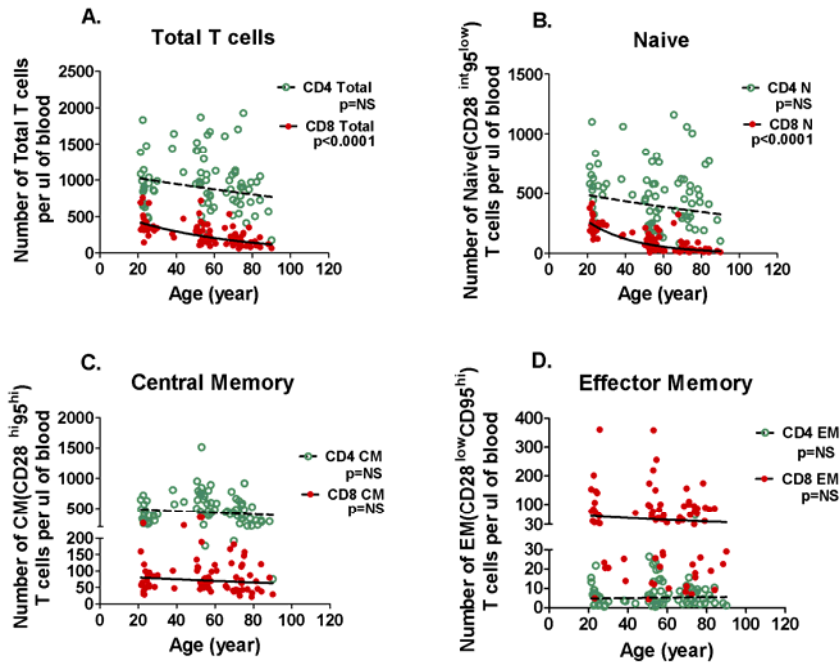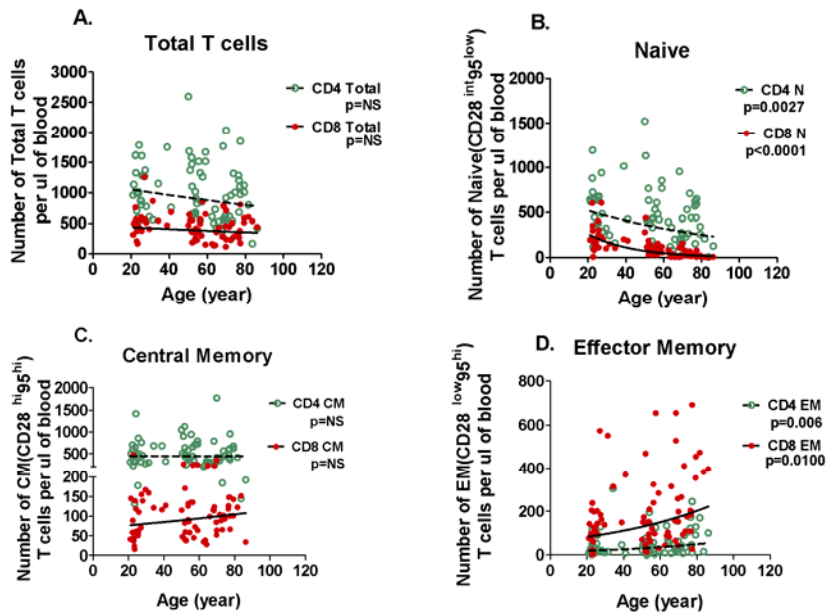

Figure S1:
Absolute numbers of CD8 T cells and CD8 naïve cells decline with age in CMV(-) subjects, memory pools accumulate with age in CMV(+) subjects – frozen PBMC analysis. Frozen PBMC were phenotyped via multi-color flow cytometry. The analysis was performed and the panels were modeled exactly as in Fig. 2 (Panel A) and Fig 3 (Panel B).

Analysis encompassed N = 78 CMV(-) individuals ranging in age from 21-100 (Panel A). Analysis encompassed N = 75 CMV(+) individuals ranging in age from 21- 96 (Panel B). The lines drawn in the figure and p values are based upon the regression parameters as detailed in the methods and illustrated within supplemental Table ST1).

Supplemental Table 1 – REGRESSION TABLES FOR FIGURES 1-3 & S1

A. Fig 2,3 Regression Table Base on the absolute cell count (log10 transformed).

Cell population	CMV Status	Regression parameter estimate Intercept(a)	Slope (Age)(b)	p-value for slope	P-value for Additivity	Final Formula for Regression Line[1] $(Y = 10^{\wedge}(a+b*X))$
CD4 Total	ALL	3.039	-0.0015	0.0247		
	CMV-	2.980	-0.0006	0.5911	0.2312	$Log_{10}(CD4T) = 3.039 - 0.0015 \times Age$
	CMV+	3.092	-0.0022	0.0091		
CD4 CM	ALL	2.478	0.0009	0.4109		
	CMV-	2.370	0.0025	0.1480	0.1676	$Log_{10}(CD4CM) = 2.478 + 0.0009 \times Age$
	CMV+	2.576	-0.0005	0.6936		
CD4 EM	ALL	0.650	0.0075	0.0001		
	CMV-	0.462	0.0025	0.3750	0.5796	CMV-: $Log_{10}(CD4EM) = 0.462 + 0.0025 \times Age$ CM+-: $Log_{10}(CD4EM) = 1.103 + 0.0044 \times Age$
	CMV+	1.103	0.0044	0.0437		
CD4 NA	ALL	2.758	-0.0025	0.0182		
	CMV-	2.714	-0.0012	0.4697	0.4270	$Log_{10}(CD4NA) = 2.758 - 0.0025 \times Age$
	CMV+	2.776	-0.0030	0.0282		
CD8 Total	ALL	2.591	-0.0037	<.0001		
	CMV-	2.664	-0.0073	<.0001	0.0084	CMV-: $Log_{10}(CD8T) = 2.664 - 0.0073 \times Age$ CMV+: $Log_{10}(CD8T) = 2.616 - 0.0029 \times Age$
	CMV+	2.616	-0.0029	0.0049		
CD8 CM	ALL	1.699	0.0019	0.0726		
	CMV-	1.725	0.0002	0.8897	0.3899	$Log_{10}(CD8CM) = 1.699 + 0.0019 \times Age$
	CMV+	1.721	0.0021	0.1179		
CD8 EM	ALL	1.597	0.0032	0.0193		
	CMV-	1.655	-0.0034	0.1030	0.0086	CMV-: $Log_{10}(CD8EM) = 1.655 - 0.0034 \times Age$ CMV+: $Log_{10}(CD8EM) = 1.752 + 0.0036 \times Age$
	CMV+	1.752	0.0036	0.0287		
CD8 NA	ALL	2.749	-0.0175	<.0001		
	CMV-	2.626	-0.0155	<.0001	0.1410	$Log_{10}(CD8NA) = 2.749 - 0.0175 \times Age$
	CMV+	2.853	-0.0191	<.0001		

1. If either additivity (equal slope) test or equal intercept test, is significant (p-value < 0.05), then separate regression model for CMV- and CMV+.

B. Fig 1 Regression Table Based on % cell population.

Cell population	CMV Status	Regression parameter estimate Intercept(a)	Slope (Age)(b)	p-value for slope	P-value for Additivity	Final Formula for Regression Line[1] $(Y = a+b*X)$
CD4 Total	ALL	67.376	0.0913	0.0006		
	CMV-	62.382	0.2599	<.0001	<0.0001	CMV-: %CD4T = 62.382 + 0.2599×Age CMV+: %CD4T = 68.477 + 0.0345×Age
	CMV+	68.477	0.0345	0.2813		
CD4 CM	ALL	36.477	0.1039	0.0192		
	CMV-	32.718	0.1572	0.0315	0.2736	%CD4CM = 36.477 + 0.1039×Age
	CMV+	39.973	0.0555	0.3339		
CD4 EM	ALL	2.281	0.0397	0.0896		
	CMV-	3.027	-0.0205	0.5864	0.1694	%CD4EM = 2.281 + 0.0397×Age
	CMV+	3.387	0.0455	0.1260		
CD4 NA	ALL	58.769	-0.1288	0.0063		
	CMV-	61.905	-0.1194	0.1196	0.7190	%CD4NA = 258.769 – 0.1288×Age
	CMV+	53.899	-0.0843	0.1628		
CD8 Total	ALL	23.071	-0.0355	0.1268	<0.0001	CMV-: %CD8T = 27.834 – 0.1936×Age CMV+: %CD8T = 21.919 + 0.0189×Age
	CMV-	27.834	-0.1936	<.0001		

		CMV+	21.919	0.0189	0.5037	
	CM	ALL	14.119	0.2949	<.0001	
		CMV-	11.686	0.4223	<.0001	0.0503
		CMV+	13.019	0.2707	<.0001	%CD8CM = 14.119 + 0.2949×Age
	EM	ALL	6.589	0.4675	<.0001	
		CMV-	11.947	0.2068	0.0075	0.0014
		CMV+	8.296	0.5222	<.0001	CMV-: %CD8EM = 11.947 + 0.2068×Age CMV+: %CD8EM = 8.296 + 0.5222×Age
	NA	ALL	77.193	-0.7572	<.0001	
		CMV-	74.843	-0.6299	<.0001	0.1222
		CMV+	75.977	-0.7802	<.0001	%CD8NA = 77.193 − 0.7572×Age

C. Supplemental Fig 1, Regression Table. Base on the absolute cell count (log10 transformed).

	Cell population	CMV Status	Regression parameter estimate		p-value for slope	P-value for Additivity	Final Formula for Regression Line' (Y = 10^(a+b*X))
			Intercept(a)	Slope (Age)(b)			
CD4	Total	ALL	3.0555	-0.00189	0.0159		
		CMV-	3.0478	-0.00179	0.1066	0.9129	Log_{10}(CD4T) = 3.0555 − 0.00189×Age
		CMV+	3.0621	-0.00197	0.0774		
	CM	ALL	2.6853	-0.00076	0.3769		
		CMV-	2.7180	-0.00138	0.2578	0.4703	Log_{10}(CD4CM) = 2.6853 − 0.00076×Age
		CMV+	2.6525	-0.00014	0.9170		
	EM	ALL	0.9462	0.00275	0.2498		
		CMV-	0.6680	0.00091	0.7235	0.0874	CMV-: Log_{10}(CD4EM) = 0.6680 + 0.00091×Age CM+-: Log_{10}(CD4EM) = 1.1124 + 0.00715×Age
		CMV+	1.1124	0.00715	0.0060		
	NA	ALL	2.7761	-0.00378	0.0021		
		CMV-	2.7374	-0.00253	0.1404	0.2697	Log_{10}(CD4NA) = 2.7761 − 0.00378×Age
		CMV+	2.8203	-0.00521	0.0027		
CD8	Total	ALL	2.7417	-0.00504	<0.0001		
		CMV-	2.7906	-0.00785	<0.0001	0.0002	CMV-: Log_{10}(CD8T) = 2.7906 − 0.00785×Age CMV+: Log_{10}(CD8T) = 2.6685 − 0.00153×Age
		CMV+	2.6685	-0.00153	0.1920		
	CM	ALL	1.8924	0.00022	0.8361		
		CMV-	1.9347	-0.00147	0.3238	0.0784	Log_{10}(CD8CM) = 1.8924 + 0.00022×Age
		CMV+	1.8394	0.00225	0.1314		
	EM	ALL	1.8390	0.00109	0.5884		
		CMV-	1.8373	-0.00289	0.2509	0.0087	CMV-: Log_{10}(CD8EM) = 1.8373 − 0.00289×Age CMV+: Log_{10}(CD8EM) = 1.7842 + 0.00654×Age
		CMV+	1.7842	0.00654	0.0100		
	NA	ALL	2.7764	-0.01764	<0.0001		
		CMV-	2.7744	-0.01730	<0.0001	0.7809	Log_{10}(CD8NA) = 2.7764 − 0.01764×Age
		CMV+	2.7825	-0.01808	<0.0001		

[1] If either additivity (equal slope) test or equal intercept test, is significant (p-value < 0.05), then separate regression model for CMV- and CMV+.
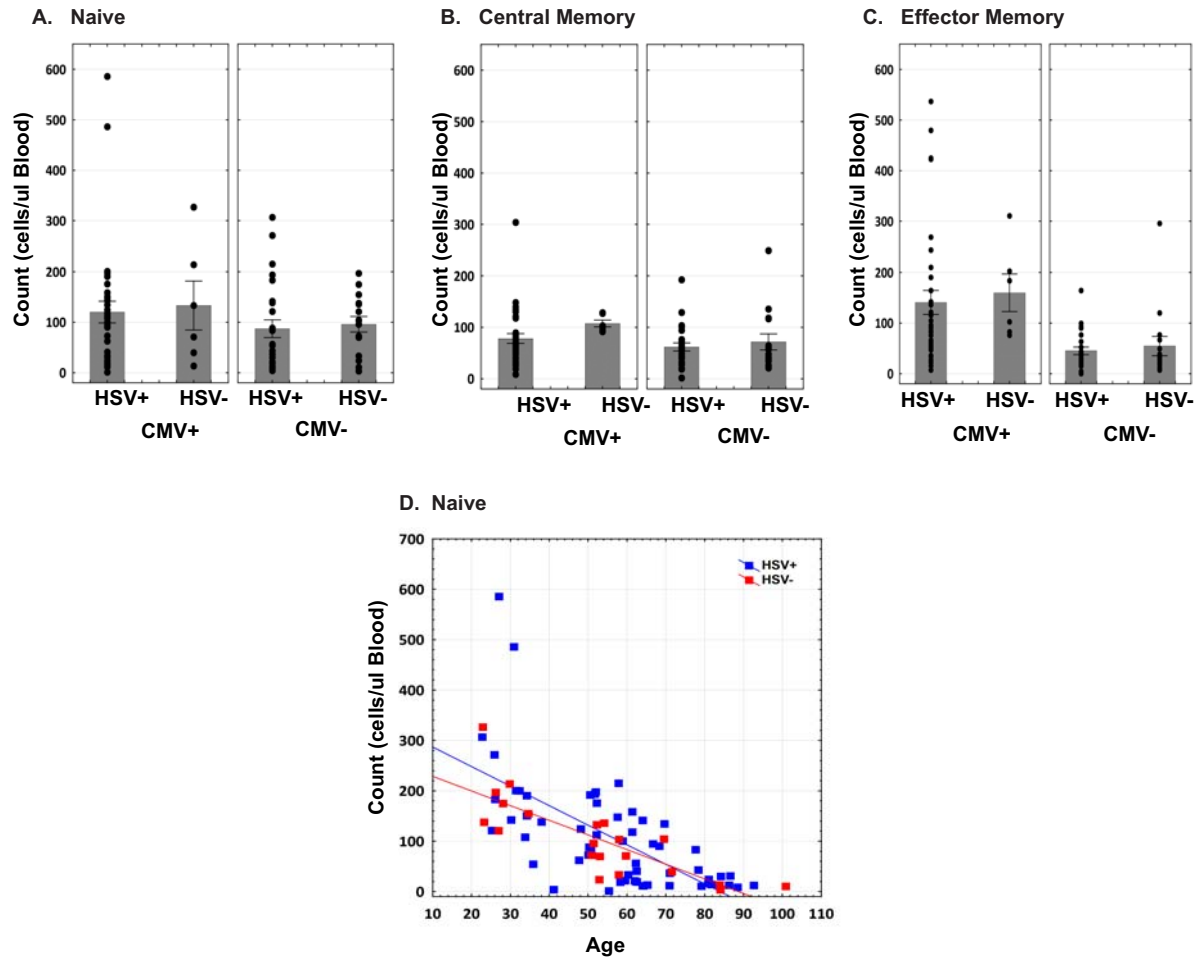
SUPPLEMENTAL FIGURE 2.

A. Naive   **B. Central Memory**   **C. Effector Memory**

D. Naive

Figure S2. **HSV-1 does not affect memory inflation.** CD8 T cell subset analysis from 80 subjects (40 CMV+ and 40 CMV-) randomly selected across the age range of the cohort (21-100yrs), were assessed based upon HSV-1 and CMV status demonstrates that naïve count (**A**) central memory count (**B**) and effector memory count (**C**) are not significantly different in the presence or the absence of HSV-1. Panel **D** illustrates that the naïve decline is also not significantly different between HSV-1 (+) and HSV-1 (-) donors over the cohort age range.
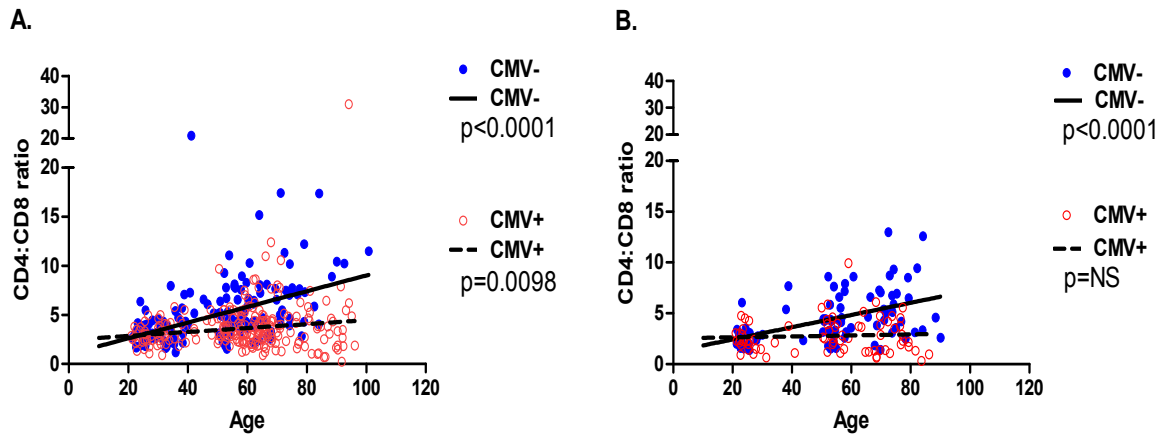
Supplemental Figure 3.

A.

B.

Figure S3. Age-related alteration of the CD4:CD8 ratio is dependent upon CMV Status.
A) Data were derived from the primary results shown in Figs. 2 & 3 and were obtained by dividing the numbers of CD4 cells by the numbers of CD8 cells for each CMV+ and CMV- individual (n=394 (CMV(-) n=152; CMV+ n=239); multiple regression model with age and CMV status as covariates was used to calculate significance. B) Frozen PBMC (n = 153 individuals (CMV(-) n=78; CMV(+) n=75) were phenotyped via multi-color flow cytometry analysis encompassing individuals between ages 21- 100.