

Supplemental figures:

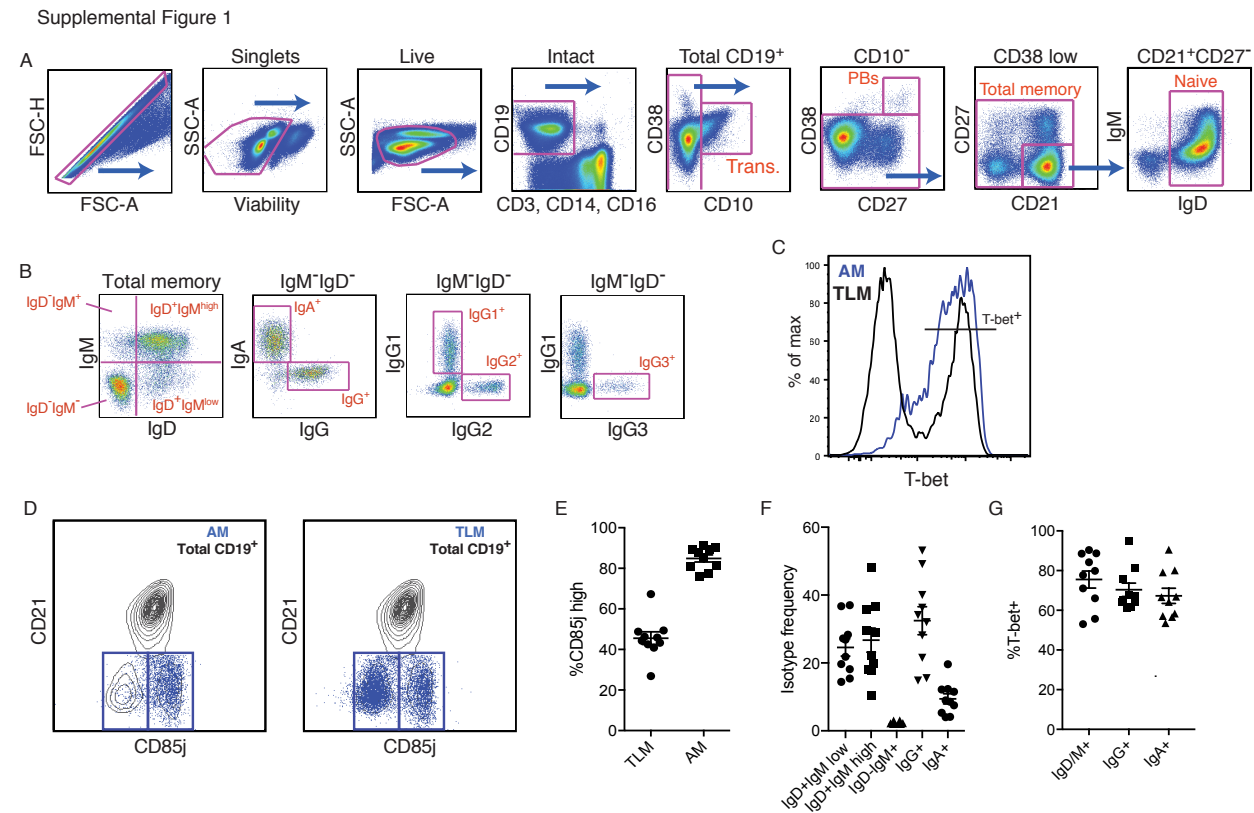


Figure 1:

(A) Representative gating scheme for the identification of B cell subsets named in red from a healthy human donor. Trans., transitional, defined as $CD19^+CD10^+CD38^{mid}CD27^-$; PBs, plasmablasts, defined as $CD19^+CD10^-CD38^{high}CD27^{high}$; naïve, defined as $CD19^+CD10^-CD38^{low}CD21^+CD27^-IgD^+$; total memory cells, defined as $CD19^+CD10^-CD38^{low}$ excluding the naïve ($CD21^+CD27^-$) population. (B) Representative identification of memory B cell surface-expressed immunoglobulin isotypes named in red. (C) AM (blue) and TLM (black) T-bet expression histogram from a representative donor. (D) Flow cytometry plots depicting the gating of AM and TLM into $CD85j^{high}$ and $CD85j^{low}$ populations. Total B cells are depicted in black contour plot; TLM/AM subsets are depicted in blue dot plot. (E) Graph depicts the frequency of AM and TLM subsets demonstrating the $CD85j^{high}$ phenotype (n=10 donors). (F) Frequency of antibody isotypes expressed by T-bet^{high} $CD85j^{high}$ cells from cohort of HIV-negative donors (n=10). (G) T-bet expression frequency of T-bet^{high} $CD85j^{high}$ cells separated by Ig isotype (n=10). No statistical differences were observed between isotypes using repeated measures one-way ANOVA.

Supplemental Figure 2

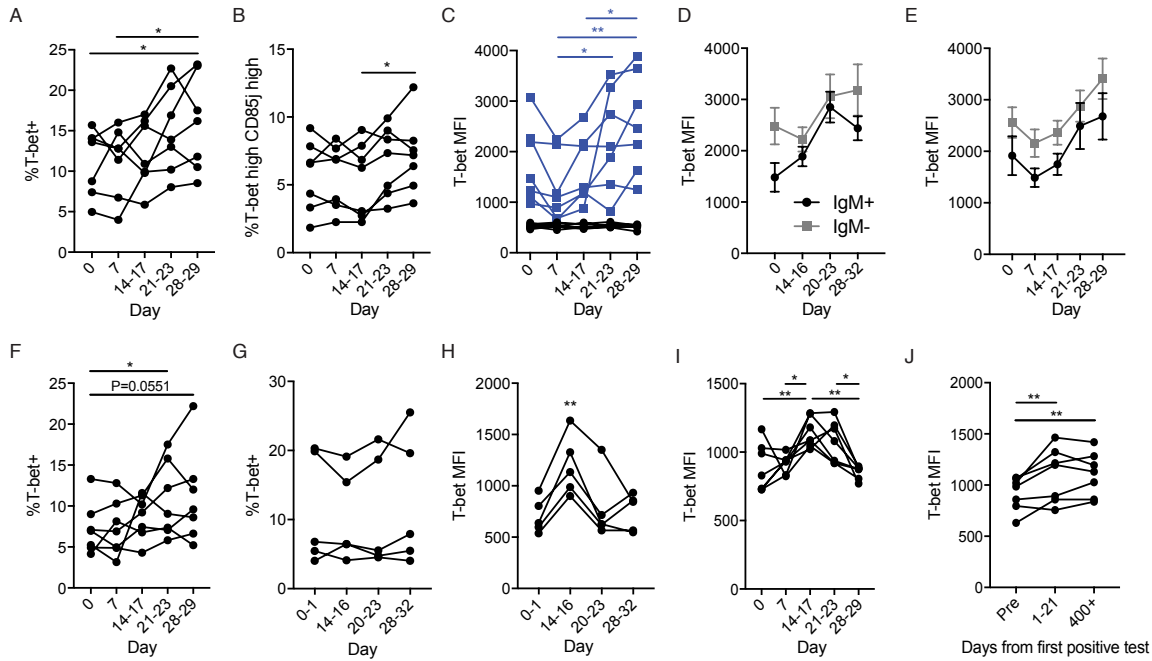


Figure 2:

(A) T-bet expression frequency of memory B cells in vaccinia vaccinees (n=7). Donor samples were binned to organize approximate weekly time points. (B) T-bet^{high}CD85j^{high} cell frequency of total memory B cells in vaccinia vaccinees. (C) T-bet MFI of T-bet^{high}CD85j^{high} cells (blue) and resting memory (RM) cells (black) from vaccinia vaccinees. No statistical differences were observed between RM time points. T-bet MFI of T-bet^{high}CD85j^{high} cells separated by IgM expression are depicted in (D) for yellow fever and (E) for vaccinia. Frequencies of RM cells expressing T-bet during VV (F) and YFV (G) responses are shown. No significant differences were observed in G between time points. (H) T-bet MFI of plasmablasts following yellow fever vaccination. (I) T-bet MFI of plasmablasts following vaccinia vaccination. (J) T-bet MFI of plasmablasts during acute and chronic HIV infection. Statistical comparisons in A, B, C, F, G, H, I, and J calculated using repeated measures ANOVA with Tukey's multiple comparisons test. * denotes a P value $0.05 > P \geq 0.01$. ** denotes a P value $0.01 > P \geq 0.001$.

Supplemental figure 3

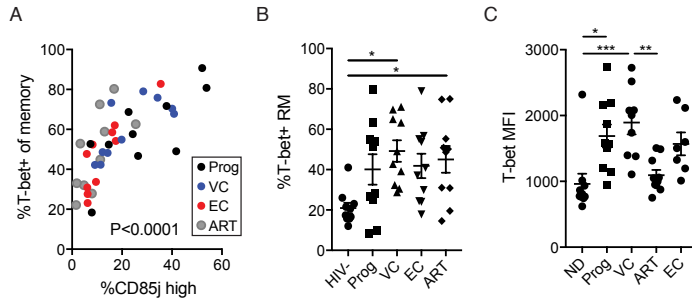


Figure 3:

(A) Correlation of total T-bet expression and T-bet^{high}CD85j^{high} cell frequencies of memory B cells. Four HIV⁺ cohorts are represented by different colors: Progressors, Prog, black; Viremic controllers, VC, blue; Elite controllers, EC, red; Aviremic individuals on antiretroviral therapy, ART, gray. Statistics were calculated using Spearman correlation. (B) Frequency of RM cells expressing T-bet by cohort. (C) T-bet MFI of plasmablasts by cohort. Statistical comparisons in B and C calculated using one-way ANOVA with Tukey's multiple comparisons test. * denotes a P value 0.05 > P ≥ 0.01. ** denotes a P value 0.01 > P ≥ 0.001. *** denotes a P value < 0.001.

Supplemental Figure 4

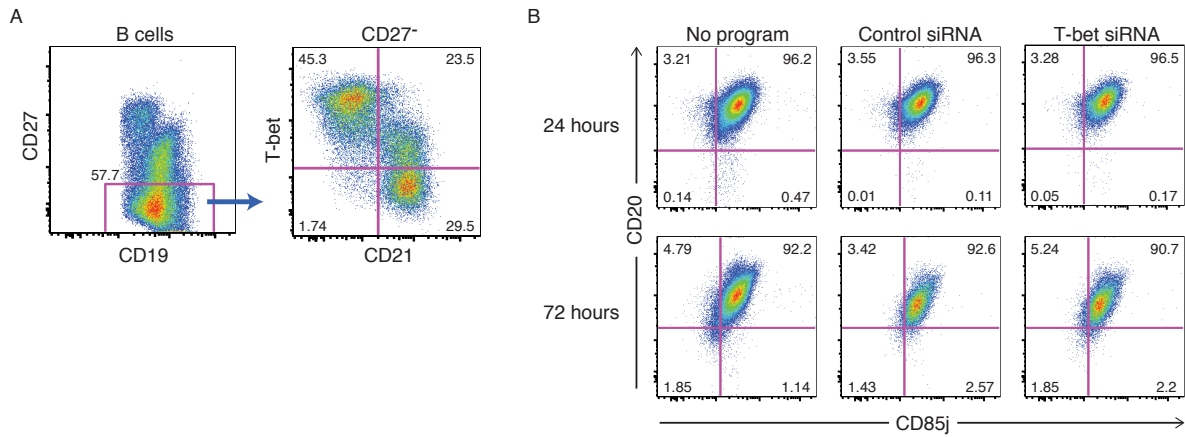


Figure 4: (A) Expression of CD21 and T-bet within CD27⁻ B cells from a representative progressor used for CD21⁻CD27⁻ B cell sorting. (B) CD85j expression by sorted CD21⁻CD27⁻ B cells at 24 and 72 hours post-nucleofection with either control or T-bet siRNA, compared to non-nucleofected sample.

Supplemental tables:

Donor ID	Group	VL	CD4 ct.	Figures
110029	ART	Undetectable	800	3B, 3C
110564	ART	Undetectable	1091	3B, 3C
110595	ART	Undetectable	702	3B, 3C
110600	ART	Undetectable	828	3B, 3C
110657	ART	Undetectable	1188	3B, 3C
110869	ART	Undetectable	679	3B, 3C
110870	ART	Undetectable	693	3B, 3C
110887	ART	Undetectable	689	3B, 3C
111031	ART	Undetectable	338	3B, 3C
111096	ART	Undetectable	686	3B, 3C
110015	ART	Undetectable	698	5C
110029	ART	Undetectable	624	5C
110043	ART	Undetectable	564	5C
110054	ART	Undetectable	644	5C
110163	ART	Undetectable	640	5C
110206	ART	Undetectable	423	5C
110285	ART	Undetectable	481	5C
110313	ART	Undetectable	804	5C
110334	ART	Undetectable	431	5C
110348	ART	Undetectable	1438	5C
110351	ART	Undetectable	382	5C
110399	Prog.	141958	70	3B, 3C
110670	Prog.	198018	248	3B, 3C
110939	Prog.	97259	250	3B, 3C
110967	Prog.	224078	8	3B, 3C
111005	Prog.	41679	221	3B, 3C
111031	Prog.	500000	90	3B, 3C
111081	Prog.	280428	222	3B, 3C
111089	Prog.	339292	377	3B, 3C
111112	Prog.	466000	5	3B, 3C
111154	Prog.	205176	8	3B, 3C
110187	Prog.	14573	393	3E, 3F, 5A
110245	Prog.	10210	216	3E, 3F, 5A
110360	Prog.	25320	349	3E, 3F, 5A
110554	Prog.	41600	777	3E, 3F, 5A
110622	Prog.	57465	471	3E, 3F, 5A
110673	Prog.	28000	387	3E, 3F, 5A
110763	Prog.	25876	270	3E, 3F, 5A
110979	Prog.	*Undetectable	1323	3E, 3F, 5A
111056	Prog.	145654	494	3E, 3F, 5A
111083	Prog.	17284	323	3E, 3F, 5A
111117	Prog.	33940	364	3E, 3F, 5A
130236	Prog.	28888	632	3D
130636	Prog.	35160	323	3D
130716	Prog.	99562	19	4C, 4E

Table 1: University of Pennsylvania Center for AIDS Research donor samples. Viral loads and CD4 counts for chronically infected HIV+ cohorts, including antiretroviral therapy (ART)-treated and ART-naïve, viremic individuals (Progressors), are depicted. The individual marked with * had undetectable viremia at the time of collection but had a viral load of 18,083 at the subsequent clinic visit 20 months later.

Donor ID	Group	VL	CD4 ct.	Figures
1161	VC	360	1185	3B, 3C, 5C, 5E, 5F
1176	EC	<40	757	3B, 3C, 5C, 5E, 5F
1203	EC	<40	890	3B, 3C, 5C, 5E, 5F
1204	EC	<40	1032	3B, 3C, 5C, 5E, 5F
1215	VC	1183	1747	3B, 3C, 5C, 5E, 5F
1247	VC	1102	591	3B, 3C, 5C, 5E, 5F
1277	VC	76.3	1074	3B, 3C, 5C, 5E, 5F
1367	EC	<40	566	3B, 3C, 5C, 5E, 5F
1446	VC	305	1086	3B, 3C, 5C, 5E, 5F
1448	VC	1400	485	3B, 3C, 5C, 5E, 5F
1508	VC	201	427	3B, 3C, 5C, 5E, 5F
1526	EC	<40	531	3B, 3C, 5C, 5E, 5F
1527	EC	<40	1085	3B, 3C, 5C, 5E, 5F
1541	VC	1589	1174	3B, 3C, 5C, 5E, 5F
1545	VC	182	625	3B, 3C, 5C, 5E, 5F
1555	EC	<40	613	3B, 3C, 5C, 5E, 5F
1581	EC	<40	1227	3B, 3C, 5C, 5E, 5F
1637	VC	676	909	3B, 3C, 5C, 5E, 5F
3604	EC	<40	912	3B, 3C, 5C, 5E, 5F
4014	EC	<40	982	3B, 3C, 5C, 5E, 5F

Table 2: University of California San Francisco SCOPE cohort. Viral loads and CD4 counts for viremic controllers (VL 41-1800 copies/ml) and elite controllers (VL <40 copies/ml) are depicted. All individuals were therapy-naïve at time of sampling.

Donor ID	Cohort	Duration	Status	VL	CD4 ct.	Figures
OM10	Toronto	>1 year	Chronic	22415	132	5C, 5E, 5F
OM17	Toronto	<6 months	Early	440000	660	5C, 5E, 5F
OM255	Toronto	<6 months	Early	18322	370	3D
OM284	Toronto	<3 months	Acute	170693	430	5C, 5E, 5F
OM5007	Toronto	<3 months	Acute	24019	440	5C, 5E, 5F
OM5031	Toronto	<1 year	Chronic	13537	330	3D, 5C, 5E, 5F
OM5037	Toronto	<3 months	Acute	13948	780	5C, 5E, 5F
OM5062	Toronto	>6 months	Chronic	187361	410	5C, 5E, 5F
OM5220	Toronto	<3 months	Acute	10640	470	3D
OM5271	Toronto	<3 months	Acute	25947	320	3D
P09586	UPenn CFAR	2.5 months	Acute	23990	446	3D, 4C, 4E, 5C, 5E, 5F
P09588	UPenn CFAR	>1 year	Chronic	80000	<500	3D, 4C, 4E, 5C, 5E, 5F
P09603	UPenn CFAR	67 days	Acute	442034	922	3D, 4C, 4E, 5C, 5E, 5F
P09636	UPenn CFAR	6 months	Early	43374	605	3D, 4C, 4E, 5C, 5E, 5F

Table 3: Acute and chronic HIV samples from University of Toronto and University of Pennsylvania CFAR cohorts. Viral loads and CD4 count for University of Toronto and University of Pennsylvania cohorts are depicted. The listed duration of infection is estimated: Acute samples are estimated to have been collected <3 months since infection; Early samples are between 3-6 months since infection; Chronic samples are from greater than 6 months since infection. All individuals were therapy-naïve at time of sampling.