SUPPLEMENTARY FIGURES

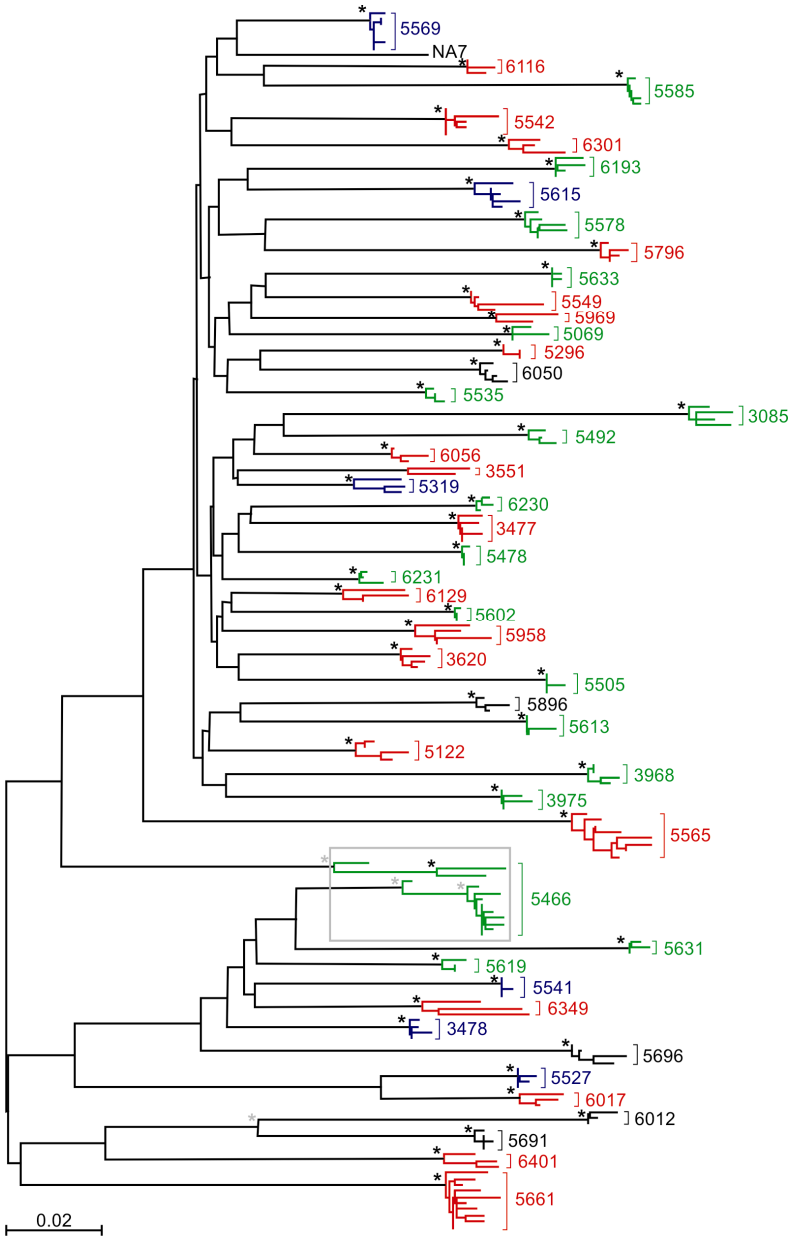

FIG. S1. Evolutionary relationships of primary HIV-1 Nef alleles. The amino acids of the newly amplified Nef alleles were aligned with NA7 (GenBank accession number DQ242535) using ClustalX (41) and amino acids were replaced by codons. Sites with a gap in any sequence were eliminated. The tree was constructed using the neighbor-joining method implemented in ClustalX using Kimura's correction and 1000 bootstrapped replicates. Black asterisks (*) on branches indicate 100% support for the cluster to the right. Grey asterisks indicate bootstrap support between 97% and 99%. Patient clusters are labeled according to their genotype and viral setpoint as defined in Fig. 1, with red indicating *-35CChi*, blue *-35CClow*, green *-35TThi* and black *-35TTlow* subjects, respectively. Sequences from patient 5466 who was coinfected with two divergent HIV-1 strains are boxed. Scale bar represents 0.02 substitutions per site.

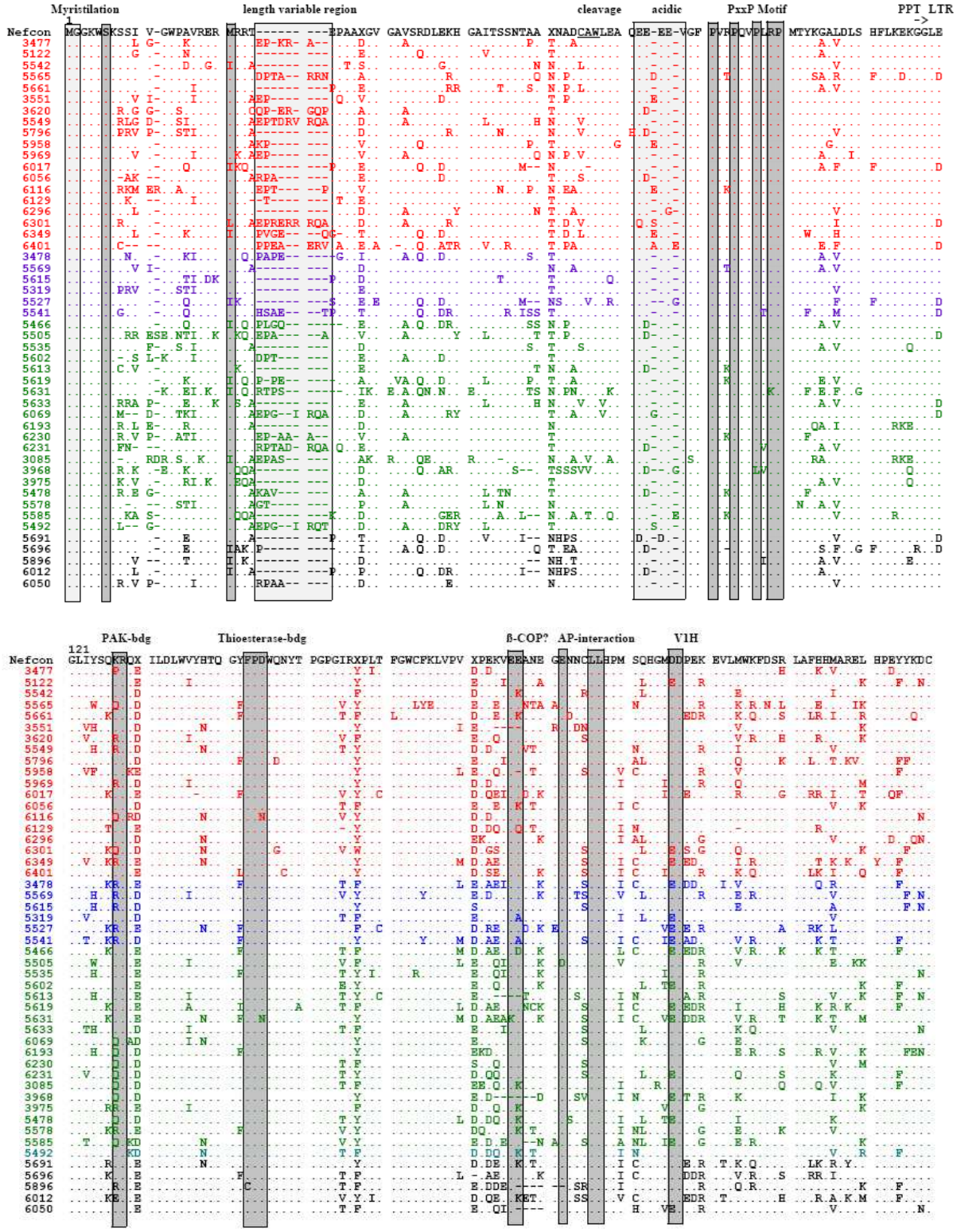

FIG. S2. Alignment of consensus HIV-1 Nef amino acid sequences. Newly derived Nef sequences are aligned with their overall consensus sequence, with dots indicating identity and dashes gaps introduced to optimize the alignment. Nef sequences are color coded as in Fig. 1, with red indicating *-35CChi*, blue *-35CClow*, green *-35TThi* and black *-35TTlow* subjects, respectively. For patient 5466, only the predominant Nef sequence is shown. Some conserved sequence elements in Nef, the position of the polypurine tract (PPT), and the start of the 3' long terminal repeat (LTR) are indicated. bdg, binding; VIH, catalytic subunit of vacuolar ATPase.

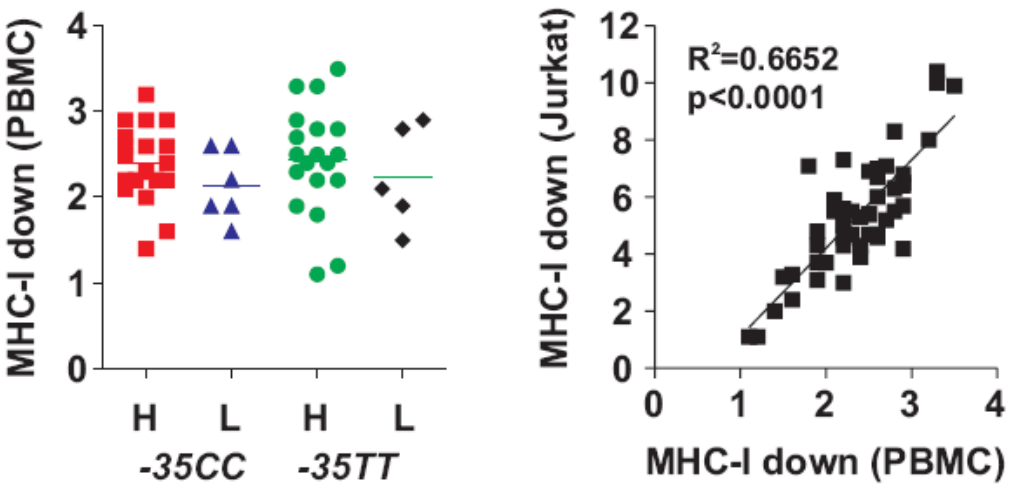

FIG. S3. Modulation of MHC-I by Nef in primary PBMCs. (Left) Quantitative assessment of Nef-mediated downmodulation of MHC-I on human PBMCs. Similar results were obtained using PBMC from a different donor. (Right) Correlation between the efficiency of Nef-mediated downmodulation of MHC-I in Jurkat T cells and PBMCs.

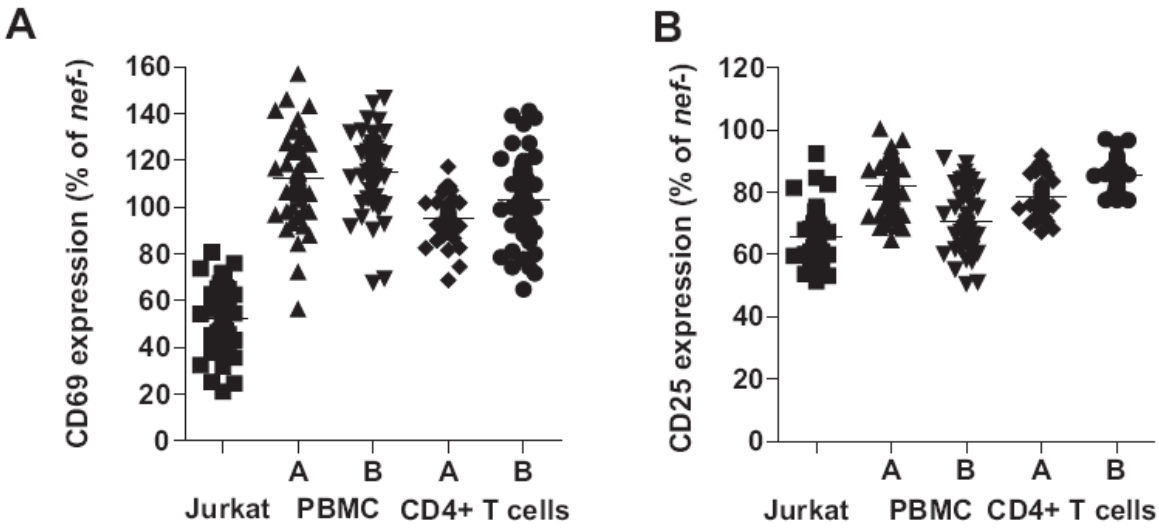

FIG. S4. Cell type dependent effect of Nef on early T cell activation. Levels of (A) CD69 and (B) CD25 expression by Jurkat cells, PBMCs and primary $CD4^+$ T cells infected with HIV-1 IRES/eGFP constructs. The expression levels are given relative to those measured for the *nef* defective control HIV-1 construct which was set to 100%. PBMCs and $CD4^+$ T cells were derived from two different donors (A and B).

TABLE S1. HLA types of HIV-1-infected individuals analyzed.

Code	Group	A1	A2	C1	C2	B1	B2	DRB1	DRB2	DQA1	DQA2	DQB1	DQB2	DPA1	DPA2	DPB1	DPB2
276	-35CC/sVLh	*2301	*3201	*0202	*0602	*4002	*5001	*0301	*1501	*0102	*0501	*0201	*0602	*0103	*0201	*0402	*1101
497	-35CC/sVLh	*2402	*3002	*0202	*0501	*1801	*2705	*0301	*1101	*0501	*0501	*0201	*0301	*0103	*0103	*0201	*0202
533	-35CC/sVLh	*6801	*6801	*0602	*0602	*4701	*5001	*0301	*0701	*0201	*0501	*0201	*0201	*0103	*0103	*0201	*1501
590	-35CC/sVLh	*0201	*2402	*0501	*0602	*3701	*4402	*0402	*0701	*0201	*0301	*0302	*0303	*0103	*0201	*0201	*0501
605	-35CC/sVLh	*0201	*3301	*0501	*0802	*1402	*4402	*0102	*0401	*0101	*0301	*0301	*0501	*0103	*0202	*0401	*0501
608	-35CC/sVLh	*0205	*3101	*0602	*1402	*5001	*5101	*0701	*0801	*0201	*0401	*0201	*0402	*0103	*0201	*0401	*1001
731	-35CC/sVLh	*0201	*0201	*0501	*0602	*4402	*5801	*0101	-	*0101	*0102	*0501	*0609	*0103	*0103	*0301	*0401
841	-35CC/sVLh	*0201	*0201	*1203	*1402	*4901	*5101	*1101	*1601	*0102	*0501	*0301	*0502	*0103	*0103	*0201	*0401
959	-35CC/sVLh	*0101	*3101	*0202	*0202	*4002	*5101	*0801	*1301	*0103	*0401	*0402	*0603	*0103	*0103	*0401	*0401
1216	-35CC/sVLh	*2402	*2902	*0303	*0602	*2705	*5001	*0401	*0701	*0201	*0301	*0201	*0302	*0103	*0201	*0401	*1401
1231	-35CC/sVLh	*3001	*3201	*0602	*1203	*1302	*3901	*0701	*0901	*0201	*0301	*0201	*0303	*0103	*0103	*0201	*0401
1402	-35CC/sVLh	*0301	*2402	*0802	*1203	*1402	*3801	*0102	*1101	*0101	*0501	*0301	*0501	*0103	*0103	*0401	*0402
1443	-35CC/sVLh	*2301	*2402	*0202	*1402	*5101	*5101	*0401	*1103	*0301	*0501	*0301	*0302	*0103	*0103	*0201	*0402
1529	-35CC/sVLh	*0101	*3201	*0602	*1203	*1302	*1801	*1501	*1601	*0102	*0102	*0502	*0602	*0103	*0103	*0301	*2301
103	-35CC/sVLl	*2301	*3201	*0202	*0602	*4002	*5001	*0301	*1501	*0102	*0501	*0201	*0602	*0103	*0201	*0402	*1101
640	-35CC/sVLl	*0301	*3301	*0802	*1203	*1402	*3901	*0102	*1601	*0101	*0102	*0501	*0502	*0103	*0201	*0401	*1001
707	-35CC/sVLl	*0201	*3002	*0102	*0802	*1402	*5101	*0102	*1501	*0101	*0102	*0501	*0602	*0103	*0103	*0201	*0201
1516	-35CC/sVLl	*3303	*6801	*0501	*1202	*4402	*5201	*1301	*1502	*0103	*0103	*0601	*0603	*0201	*0202	*0401	*1001
111	-35TT/sVLh	*0101	*2402	*0701	*0701	*0801	*1517	*0101	*1302	*0101	*0102	*0501	*0604	*0103	*0103	*0401	*0402
123	-35TT/sVLh	*0201	*0201	*0501	*0702	*0702	*5101	*0401	*0404	*0301	*0301	*0302	*0302	*0103	*0103	*0301	*0601
163	-35TT/sVLh	*0201	*3201	*0102	*1602	*5101	*5601	*1101	*1302	*0102	*0501	*0301	*0604	*0103	*0103	*0201	*0401
525	-35TT/sVLh	*1101	*2601	*0701	*0701	*0801	*1801	*0301	*1601	*0102	*0501	*0201	*0502	*0103	*0201	*0401	*1001
526	-35TT/sVLh	*2501	*2501	*0701	*0701	*0801	*0801	*0301	*0301	*0501	*0501	*0201	*0201	*0201	*0201	*0101	*0101
537	-35TT/sVLh	*2301	*2601	*0303	*1505	*0705	*1501	*0701	*1501	*0102	*0201	*0201	*0602	*0103	*0201	*0402	*1401
546	-35TT/sVLh	*0201	*0301	*0401	*0702	*0702	*3501	*0401	*1101	*0301	*0501	*0301	*0301	*0103	*0103	*0401	*0401
554	-35TT/sVLh	*0201	*0201	*0303	*0303	*1501	*1501	*0401	*0401	*0301	*0301	*0302	*0302	*0103	*0103	*0401	*0401
585	-35TT/sVLh	*0101	*0201	*0401	*0701	*0801	*5101	*0301	*0301	*0501	*0501	*0201	*0201	*0103	*0103	*0401	*0401
614	-35TT/sVLh	*0101	*0301	*0701	*0702	*0702	*0801	*0301	*0701	*0201	*0501	*0201	*0201	*0103	*0103	*0201	*0401
638	-35TT/sVLh	*0201	*1101	*0303	*0702	*0702	*5501	*1001	*1401	*0101	*0101	*0501	*0503	*0103	*0103	*0401	*0401
1171	-35TT/sVLh	*2501	*3101	*0304	*0702	*0702	*4001	*0404	*1501	*0102	*0301	*0302	*0602	*0103	*0103	*0201	*0401
1294	-35TT/sVLh	*1101	-	*0701	*1502	*1517	*5101	*0404	*1601	*0102	*0301	*0302	*0502	*0103	*0103	*0401	*0402
1332	-35TT/sVLh	*0301	*2402	*0401	*0702	*0702	*3501	*0801	*1101	*0401	*0501	*0301	*0402	*0103	*0103	*0301	*0601
1334	-35TT/sVLh	*0201	*0301	*0701	*0702	*0702	*5101	*1301	*1501	*0102	*0103	*0602	*0603	*0103	*0103	*0201	*0301
313	-35TT/sVLl	*0201	*2501	*0304	*0701	*0801	*1501	*0301	*0401	*0301	*0501	*0201	*0302	*0103	*0201	*0101	*0401
1477	-35TT/sVLl	*0201	*0301	*0304	*0401	*1501	*3501	*0101	*0401	*0101	*0301	*0302	*0501	*0103	*0201	*0402	*1001
162	-35TT/sVLl	*0301	*3002	*0304	*0701	*5703	*1510										

TABLE S2. Functional activity of HIV-1 *nef* alleles from -35TT or -35CC individuals with high or low set-point viral loads.

	Receptor modulation[a]								T cell activation[b]			
Group	MHC-I	HLA-A	HLA-B	HLA-C	CD4	CD28	CXCR4	CD74	NF-AT	CD69	Infectivity[c]	Repl.(%)[d]
-*35CC*/sVLh (n=19)	5.1±0.3	3.7±0.3	3.7±0.3	1.7±0.1	3.8±0.2	1.5±0.1	1.6±0.1	5.0±0.4	124±12	95±3	8.0±0.7	133±12
-*35CC*/sVLl (n=6)	4.6±0.6	3.5±0.5	3.6±0.5	1.8±0.2	2.9±0.2	1.2±0.1	1.4±0.1	3.1±0.7	195±21	98±5	4.8±0.6	136±32
-*35TT*/sVLh (n=19)	6.0±0.6	4.2±0.5	4.2±0.5	2.0±0.2	3.7±0.3	1.6±0.1	1.5±0.1	5.6±0.6	174±12	101±3	6.4±0.5	170±13
-*35TT*/sVLl (n=5)	4.9±0.6	3.1±0.3	3.1±0.4	1.7±0.4	4.8±0.3	1.6±0.1	1.9±0.3	5.2±1.2	218±59	107±6	6.8±1.1	165±22

[a] N-fold down-modulation of CD4, CD28, CD3, MHC-I, MHC-II and up-regulation of Ii surface expression levels were determined as described in the Materials and Methods. In all analyses at least 500 HIV-1-infected (GFP+) cells were analyzed. Shown are average values derived from three to five independent experiments (±SD).

[b] Values were determined as described in the legend to Fig. 5.

[c] Shown is the n-fold enhancement of viral infectivity compared to the *nef*-deleted HIV-1 NL4-3 control construct. Values represent the average of three measurements (±SD).

[d] The efficiency of virus spread was determined in PBMC and is shown relative to the proviral HIV-1 NL4-3 Nef (*nef*+) construct (100%)