Supplementary Information

Enhancement of anti-STLV-1/HTLV-1 immune responses through multimodal effects of anti-CCR4 antibody

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Table S1

Sequences of sTax overlap peptides used in this study.

Pep No	Name	Start	End	Sequence	Hydro	MWt
1	sTax pep1	1	20	MAHFPGFGQSLLYGYPVYVF	0.69	2293.7
2	sTax pep2	7	26	FGQSLLYGYPVYVFGDCVQG	0.58	2212.5
3	sTax pep3	13	32	YGYPVYVFGDCVQGDWCPIS	0.61	2268.6
4	sTax pep4	19	38	VFGDCVQGDWCPISGGLCSA	0.55	2014.3
5	sTax pep5	25	44	QGDWCPISGGLCSARLHRHA	0.33	2164.5
6	sTax pep6	31	50	ISGGLCSARLHRHALLATCP	0.45	2076.5
7	sTax pep7	37	56	SARLHRHALLATCPEHQITW	0.39	2340.7
8	sTax pep8	43	62	HALLATCPEHQITWDPIDGR	0.38	2273.6
9	sTax pep9	49	68	CPEHQITWDPIDGRVIGSAL	0.41	2207.5
10	sTax pep10	55	74	TWDPIDGRVIGSALQFLIPR	0.48	2254.6
11	sTax pep11	61	80	GRVIGSALQFLIPRLPSFPT	0.55	2169.6
12	sTax pep12	67	86	ALQFLIPRLPSFPTQRTSKT	0.38	2301.7
13	sTax pep13	73	92	PRLPSFPTQRTSKTLKVLTP	0.27	2267.7
14	sTax pep14	79	98	PTQRTSKTLKVLTPPATHTT	0.18	2178.5
15	sTax pep15	85	104	KTLKVLTPPATHTTPNIPPS	0.34	2113.5
16	sTax pep16	91	110	TPPATHTTPNIPPSFFQAVR	0.38	2179.5
17	sTax pep17	97	116	TTPNIPPSFFQAVRKYSPFR	0.34	2353.7
18	sTax pep18	103	122	PSFFQAVRKYSPFRNGYMEP	0.27	2421.8
19	sTax pep19	109	128	VRKYSPFRNGYMEPTLGQQL	0.22	2384.8
20	sTax pep20	115	134	FRNGYMEPTLGQQLPTLSFP	0.41	2296.6
21	sTax pep21	121	140	EPTLGQQLPTLSFPDPGLRP	0.37	2163.5
22	sTax pep22	127	146	QLPTLSFPDPGLRPQNLYTL	0.46	2270.6
23	sTax pep23	133	152	FPDPGLRPQNLYTLWGNSVV	0.45	2273.6
24	sTax pep24	139	158	RPQNLYTLWGNSVVCMYLYQ	0.54	2448.9
25	sTax pep25	145	164	TLWGNSVVCMYLYQLSPPIT	0.71	2285.7
26	sTax pep26	151	170	VVCMYLYQLSPPITWPLLPH	0.87	2370.9
27	sTax pep27	157	176	YQLSPPITWPLLPHVIFCHP	0.84	2358.8
28	sTax pep28	163	182	ITWPLLPHVIFCHPGQLGAF	0.83	2246.7
29	sTax pep29	169	188	PHVIFCHPGQLGAFLTNVPY	0.64	2210.6
30	sTax pep30	175	194	HPGQLGAFLTNVPYKRMEEL	0.30	2300.7
31	sTax pep31	181	200	AFLTNVPYKRMEELLYKIFL	0.51	2489.0
32	sTax pep32	187	206	PYKRMEELLYKIFLNTGATI	0.39	2400.9
33	sTax pep33	193	212	ELLYKIFLNTGATIILPEDC	0.57	2266.7
34	sTax pep34	199	218	FLNTGATIILPEDCLPTTLF	0.66	2179.6
35	sTax pep35	205	224	TIILPEDCLPTTLFQPTRAP	0.54	2226.6
36	sTax pep36	211	230	DCLPTTLFQPTRAPATLTAW	0.51	2203.6
37	sTax pep37	217	236	LFQPTRAPATLTAWQNGLLP	0.50	2195.6
38	sTax pep38	223	242	APATLTAWQNGLLPFQSTLT	0.52	2130.4
39	sTax pep39	229	248	AWQNGLLPFQSTLTTPGLIW	0.66	2243.6
40	sTax pep40	235	254	LPFQSTLTTPGLIWTFTDGT	0.59	2196.5
41	sTax pep41	241	260	LTTPGLIWTFTDGTPMVSGP	0.57	2091.4
42	sTax pep42	247	266	IWTFTDGTPMVSGPCPRDGQ	0.37	2165.4
43	sTax pep43	253	272	GTPMVSGPCPRDGQPSLVLQ	0.35	2039.4
44	sTax pep44	259	278	GPCPRDGQPSLVLQSSSFIF	0.43	2135.4
45	sTax pep45	265	284	GQPSLVLQSSSFIFHKFQTK	0.38	2279.6
46	sTax pep46	271	290	LQSSSFIFHKFQTKAYHPSF	0.40	2400.7
47	sTax pep47	277	296	IFHKFQTKAYHPSFLLSHGL	0.49	2371.8
48	sTax pep48	283	302	TKAYHPSFLLSHGLIQYSSF	0.49	2296.6
49	sTax pep49	289	308	SFLLSHGLIQYSSFHNLHLL	0.65	2326.7
50	sTax pep50	295	314	GLIQYSSFHNLHLLFEEYTN	0.46	2425.7
51	sTax pep51	301	320	SFHNLHLLFEEYTNIPISLL	0.62	2400.8
52	sTax pep52	307	326	LLFEEYTNIPISLLFNKEEA	0.44	2383.7
53	sTax pep53	313	332	TNIPISLLFNKEEANDTDHE	0.11	2300.5
54	sTax pep54	319	338	LLFNKEEANDTDHEPQMLPG	0.12	2298.5
55	sTax pep55	325	344	EANDTDHEPQMLPGGLKPPN	0.06	2160.4
56	sTax pep56	331	350	HEPQMLPGGLKPPNEKHFRE	0.09	2341.7
57	sTax pep57	334	353	QMLPGGLKPPNEKHFRETDV	0.12	2293.6

Table S2

Sequences of SBZ overlap peptides and vaccinia peptides used in this study.

Pep No	Name	Start	End	Sequence	Hydro	MWt
1	SBZ pep1	1	20	MAASGPFRCLPVPCPEDLLV	0.59	2115.6
2	SBZ pep2	7	26	FRCLPVPCPEDLLVDDLVDG	0.50	2215.6
3	SBZ pep3	13	32	PCPEDLLVDDLVDGLLSLEE	0.40	2184.5
4	SBZ pep4	19	38	LVDDLVDGLLSLEEDLNKQR	0.17	2284.6
5	SBZ pep5	25	44	DGLLSLEEDLNKQRTEEESV	-0.05	2304.5
6	SBZ pep6	31	50	EEDLNKQRTEEESVLDGLLS	-0.05	2304.5
7	SBZ pep7	37	56	QRTEEESVLDGLLSLEEECY	0.14	2342.5
8	SBZ pep8	43	62	SVLDGLLSLEEECYGQQQVP	0.33	2207.5
9	SBZ pep9	49	68	LSLEEECYGQQQVPLREESP	0.16	2334.6
10	SBZ pep10	55	74	CYGQQQVPLREESPPRGETY	0.11	2337.6
11	SBZ pep11	61	80	VPLREESPPRGETYRDRQRR	-0.20	2497.8
12	SBZ pep12	67	86	SPPRGETYRDRQRRAEEKRK	-0.44	2515.8
13	SBZ pep13	73	92	TYRDRQRRAEEKRKRKRERE	-0.67	2747.1
14	SBZ pep14	79	98	RRAEEKRKRKREREKEEEEQ	-0.76	2700.0
15	SBZ pep15	85	104	RKRKREREKEEEEQIAEFLR	-0.40	2660.0
16	SBZ pep16	91	110	REKEEEEQIAEFLRKKEEKK	-0.39	2576.9
17	SBZ pep17	97	116	EQIAEFLRKKEEKKARRRRR	-0.39	2628.1
18	SBZ pep18	103	122	LRKKEEKKARRRRREEEKAA	-0.61	2568.0
19	SBZ pep19	109	128	KKARRRRREEEKAAYRARRK	-0.61	2615.1
20	SBZ pep20	115	134	RREEEKAAYRARRKREEEER	-0.61	2647.9
21	SBZ pep21	121	140	AAYRARRKREEEERLERKRR	-0.52	2659.0
22	SBZ pep22	127	146	RKREEEERLERKRRLAEQGA	-0.45	2539.9
23	SBZ pep23	133	152	ERLERKRRLAEQGAKRARQR	-0.40	2507.9
24	SBZ pep24	139	158	RRLAEQGAKRARQRDTRKEK	-0.47	2453.8
25	SBZ pep25	145	164	GAKRARQRDTRKEKIKELGV	-0.29	2339.7
26	SBZ pep26	151	170	QRDTRKEKIKELGVDGYARQ	-0.22	2390.7
27	SBZ pep27	157	176	EKIKELGVDGYARQLESEVD	-0.01	2278.5
28	SBZ pep28	163	182	GVDGYARQLESEVDSLEAER	-0.03	2223.3
29	SBZ pep29	169	188	RQLESEVDSLEAERKRLLQE	-0.09	2428.7
30	SBZ pep30	175	194	VDSLEAERKRLLQEKEDLMG	-0.03	2359.7
31	SBZ pep31	181	200	ERKRLLQEKEDLMGEVNYWQ	0.03	2564.9
32	SBZ pep32	187	206	QEKEDLMGEVNYWQGRLQAM	0.13	2425.7
33	SBZ pep33	190	209	EDLMGEVNYWQGRLQAMWSQ	0.31	2441.7

Pep No	Name	Start	End	Sequence	Hydro	MWt
1	VV B8R	20	27	TSYKFESV	0.08	960.1
2	VV L2R	53	61	VIYIFTVRL	0.73	1123.4
3	VV K3L	6	15	YSLPNAGDVI	0.30	1048.2

Table S3

Sequences of Tax overlap peptides used in this study.

Pep No	Name	Start	End	Sequence	Hydro	MWt
1	Tax pep1	1	20	MAHFPGFGQSLLFGYPVYVF	0.73	2277.7
2	Tax pep2	7	26	FGQSLLFGYPVYVFGDCVQG	0.62	2196.5
3	Tax pep3	13	32	FGYPVYVFGDCVQGDWCPIS	0.64	2252.6
4	Tax pep4	19	38	VFGDCVQGDWCPISGGLCSA	0.55	2014.3
5	Tax pep5	25	44	QGDWCPISGGLCSARLHRHA	0.33	2164.5
6	Tax pep6	31	50	ISGGLCSARLHRHALLATCP	0.45	2076.5
7	Tax pep7	37	56	SARLHRHALLATCPEHQITW	0.39	2340.7
8	Tax pep8	43	62	HALLATCPEHQITWDPIDGR	0.38	2273.6
9	Tax pep9	49	68	CPEHQITWDPIDGRVIGSAL	0.41	2207.5
10	Tax pep10	55	74	TWDPIDGRVIGSALQFLIPR	0.48	2254.6
11	Tax pep11	61	80	GRVIGSALQFLIPRLPSFPT	0.55	2169.6
12	Tax pep12	67	86	ALQFLIPRLPSFPTQRTSKT	0.38	2301.7
13	Tax pep13	73	92	PRLPSFPTQRTSKTLKVLTP	0.27	2267.7
14	Tax pep14	79	98	PTQRTSKTLKVLTPPITHTT	0.25	2220.6
15	Tax pep15	85	104	KTLKVLTPPITHTTPNIPPS	0.40	2155.6
16	Tax pep16	91	110	TPPITHTTPNIPPSFLQAMR	0.44	2219.6
17	Tax pep17	97	116	TTPNIPPSFLQAMRKYSPFR	0.33	2351.8
18	Tax pep18	103	122	PSFLQAMRKYSPFRNGYMEP	0.26	2419.8
19	Tax pep19	109	128	MRKYSPFRNGYMEPTLGQHL	0.23	2425.8
20	Tax pep20	115	134	FRNGYMEPTLGQHLPTLSFP	0.43	2305.7
21	Tax pep21	121	140	EPTLGQHLPTLSFPDPGLRP	0.38	2172.5
22	Tax pep22	127	146	HLPTLSFPDPGLRPQNLYTL	0.47	2279.6
23	Tax pep23	133	152	FPDPGLRPQNLYTLWGGSVV	0.48	2216.5
24	Tax pep24	139	158	RPQNLYTLWGGSVVCMYLYQ	0.57	2391.8
25	Tax pep25	145	164	TLWGGSVVCMYLYQLSPPIT	0.74	2228.7
26	Tax pep26	151	170	VVCMYLYQLSPPITWPLLPH	0.87	2370.9
27	Tax pep27	157	176	YQLSPPITWPLLPHVIFCHP	0.84	2358.8
28	Tax pep28	163	182	ITWPLLPHVIFCHPGQLGAF	0.83	2246.7
29	Tax pep29	169	188	PHVIFCHPGQLGAFLTNVPY	0.64	2210.6
30	Tax pep30	175	194	HPGQLGAFLTNVPYKRIEEL	0.33	2282.6
31	Tax pep31	181	200	AFLTNVPYKRIEELLYKISL	0.45	2410.9
32	Tax pep32	187	206	PYKRIEELLYKISLTTGALI	0.44	2321.8
33	Tax pep33	193	212	ELLYKISLTTGALIILPEDC	0.60	2205.7
34	Tax pep34	199	218	SLTTGALIILPEDCLPTTLF	0.68	2118.5
35	Tax pep35	205	224	LIILPEDCLPTTLFQPARAP	0.61	2208.7
36	Tax pep36	211	230	DCLPTTLFQPARAPVTLTAW	0.56	2201.6
37	Tax pep37	217	236	LFQPARAPVTLTAWQNGLLP	0.54	2193.6
38	Tax pep38	223	242	APVTLTAWQNGLLPFHSTLT	0.58	2167.5
39	Tax pep39	229	248	AWQNGLLPFHSTLTTPGLIW	0.68	2252.6
40	Tax pep40	235	254	LPFHSTLTTPGLIWTFTDGT	0.60	2205.5
41	Tax pep41	241	260	LTTPGLIWTFTDGTPMISGP	0.60	2105.4
42	Tax pep42	247	266	IWTFTDGTPMISGPCPKDGQ	0.40	2151.5
43	Tax pep43	253	272	GTPMISGPCPKDGQPSLVLQ	0.38	2025.4
44	Tax pep44	259	278	GPCPKDGQPSLVLQSSSFIF	0.44	2107.4
45	Tax pep45	265	284	GQPSLVLQSSSFIFHKFQTK	0.38	2279.6
46	Tax pep46	271	290	LQSSSFIFHKFQTKAYHPSF	0.40	2400.7
47	Tax pep47	277	296	IFHKFQTKAYHPSFLLSHGL	0.49	2371.8
48	Tax pep48	283	302	TKAYHPSFLLSHGLIQYSSF	0.49	2296.6
49	Tax pep49	289	308	SFLLSHGLIQYSSFHNLHLL	0.65	2326.7
50	Tax pep50	295	314	GLIQYSSFHNLHLLFEEYTN	0.46	2425.7
51	Tax pep51	301	320	SFHNLHLLFEEYTNIPISLL	0.62	2400.8
52	Tax pep52	307	326	LLFEEYTNIPISLLFNEKEA	0.44	2383.7
53	Tax pep53	313	332	TNIPISLLFNEKEADDNDHE	0.06	2314.5
54	Tax pep54	319	338	LLFNEKEADDNDHEPQISPG	0.02	2268.4
55	Tax pep55	325	344	EADDNDHEPQISPGGLEPPS	0.00	2104.1
56	Tax pep56	331	350	HEPQISPGGLEPPSEKHFRE	0.08	2271.5
57	Tax pep57	334	353	QISPGGLEPPSEKHFRETEV	0.11	2237.5

Table S4

Sequences of HBZ overlap peptides used in this study.

Pep No	Name	Start	End	Sequence	Hydro	MWt
1	HBZ pep1	1	20	MAASGLFRCLPVPCPEDLLV	0.63	2131.6
2	HBZ pep2	7	26	FRCLPVPCPEDLLVEELVDG	0.51	2243.6
3	HBZ pep3	13	32	PCPEDLLVEELVDGLLSLEE	0.29	2128.3
4	HBZ pep4	19	38	LVEELVDGLLSLEEELKDKE	0.03	2216.4
5	HBZ pep5	25	44	DGLLSLEEELKDKEEEEAVL	-0.12	2204.3
6	HBZ pep6	31	50	EEELKDKEEEEAVLDGLLSL	0.01	2288.5
7	HBZ pep7	37	56	KEEEEAVLDGLLSLEEESRG	-0.03	2232.4
8	HBZ pep8	43	62	VLDGLLSLEEESRGRLRRGP	0.09	2252.6
9	HBZ pep9	49	68	SLEEESRGRLRRGPPGEKAP	-0.16	2221.5
10	HBZ pep10	55	74	RGRLRRGPPGEKAPPRGETH	-0.17	2224.5
11	HBZ pep11	61	80	GPPGEKAPPRGETHRDRQRR	-0.30	2297.5
12	HBZ pep12	67	86	APPRGETHRDRQRRAEEKRK	-0.47	2473.8
13	HBZ pep13	73	92	THRDRQRRAEEKRKRKKERE	-0.71	2693.0
14	HBZ pep14	79	98	RRAEEKRKRKKEREKEEEKQ	-0.78	2671.0
15	HBZ pep15	85	104	RKRKKEREKEEEKQIAEYLK	-0.46	2619.0
16	HBZ pep16	91	110	REKEEEKQIAEYLKRKEEEK	-0.42	2592.9
17	HBZ pep17	97	116	KQIAEYLKRKEEEKARRRRR	-0.43	2644.1
18	HBZ pep18	103	122	LKRKEEEKARRRRRAEKKAA	-0.56	2510.0
19	HBZ pep19	109	128	EKARRRRRAEKKAADVARRK	-0.55	2451.9
20	HBZ pep20	115	134	RRAEKKAADVARRKQEEQER	-0.50	2454.7
21	HBZ pep21	121	140	AADVARRKQEEQERRERKWR	-0.42	2597.9
22	HBZ pep22	127	146	RKQEEQERRERKWRQGAEKA	-0.49	2598.9
23	HBZ pep23	133	152	ERRERKWRQGAEKAKQHSAR	-0.40	2507.8
24	HBZ pep24	139	158	WRQGAEKAKQHSARKEKMQE	-0.26	2426.8
25	HBZ pep25	145	164	KAKQHSARKEKMQELGIDGY	-0.13	2317.7
26	HBZ pep26	151	170	ARKEKMQELGIDGYTRQLEG	-0.04	2322.6
27	HBZ pep27	157	176	QELGIDGYTRQLEGEVESLE	0.10	2265.4
28	HBZ pep28	163	182	GYTRQLEGEVESLEAERRKL	-0.06	2363.6
29	HBZ pep29	169	188	EGEVESLEAERRKLLQEKED	-0.21	2387.6
30	HBZ pep30	175	194	LEAERRKLLQEKEDLMGEVN	-0.05	2400.7
31	HBZ pep31	181	200	KLLQEKEDLMGEVNYWQGRL	0.18	2449.8
32	HBZ pep32	187	206	EDLMGEVNYWQGRLEAMWLQ	0.37	2468.8

Table S5

Clinical information of mogamulizumab-treated ATL patients

Patient ID	Disease status at analysis	Number of Mogamulizumab administration (times)	Time after Mogamulizumab treatment (weeks)
CR1	CR	5	87
CR2	CR	4	13
CR3	CR	8	44
CR4	CR	6	173
CR5	CR	5	56
SD6	SD	3	11
SD7	SD	3	7
SD8	SD	8	9
PD9	PD	1	3
PD10	PD	1	26

Table S6

ELISPOT data in ATL and HAM/TSP patients.

HAM	IFN-γ spots/10^5 PBMCs		
Pt. No.	Tax-PA	Tax-PB	Total
HAM#1	4	20	24
HAM#2	6	10	16
HAM#3	3	4	7
HAM#4	9	25	34
HAM#5	92	3	95
HAM#6	49	1	50
HAM#7	1	4	5
HAM#8	0	28	28

IFN-γ spots/10^5 PBMCs		
HBZ-PA	HBZ-PB	Total
1	0	1
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
1	0	1

Mogamulizumab-ATL	IFN-γ spots/10^5 PBMCs		
Pt. No.	Tax-PA	Tax-PB	Total
CR#1	5	1	6
CR#2	9	60	69
CR#3	32	20	52
CR#4	2	16	18
CR#5	0	0	0
SD#6	0	0	0
SD#7	0	0	0
SD#8	2	1	3
PD#9	0	0	0
PD#10	0	0	0

IFN-γ spots/10^5 PBMCs		
HBZ-PA	HBZ-PB	Total
0	0	0
1	0	1
5	1	6
1	1	2
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0
0	0	0

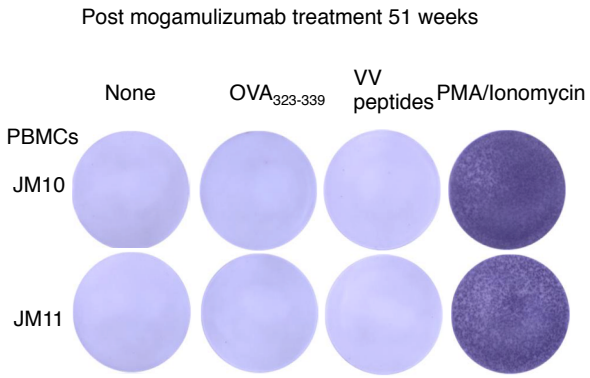
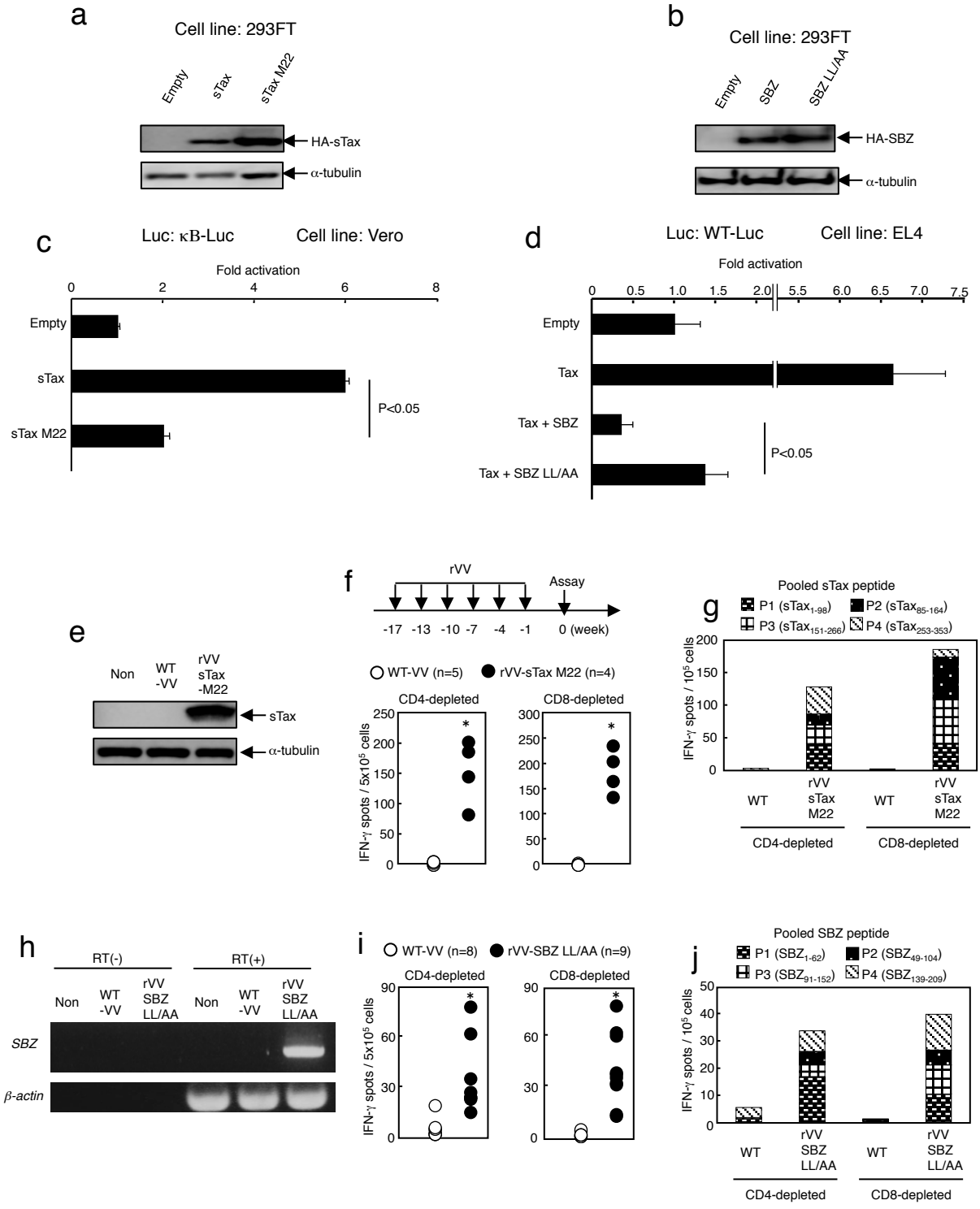


Figure S1 Positive and negative controls in IFN-γ ELISPOT assay of monkey PBMCs.

Monkey PBMCs were stimulated with $OVA_{323-339}$ and VV peptides (T cell epitopes in $H\text{-}2^b$ (C57BL/6) haplotypes) as negative control. We stimulated T cells by PMA and ionomycin as a positive control.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Cell line: 293FT
Empty
sTax
sTax M22
HA-sTax
α-tubulin
b
Cell line: 293FT
Empty
SBZ
SBZ LL/AA
HA-SBZ
α-tubulin
c
Luc: κB-Luc
Cell line: Vero
Fold activation
0
2
4
6
8
Empty
sTax
sTax M22
P<0.05
d
Luc: WT-Luc
Cell line: EL4
Fold activation
0
0.5
1.0
1.5
2.0
5.5
6.0
6.5
7.0
7.5
Empty
Tax
Tax + SBZ
Tax + SBZ LL/AA
P<0.05
e
Non
WT -VV
rVV sTax -M22
sTax
α-tubulin
f
rVV
Assay
-17 -13 -10 -7 -4 -1 0 (week)
WT-VV (n=5)
rVV-sTax M22 (n=4)
CD4-depleted
CD8-depleted
IFN-γ spots / 5x10^5 cells
g
Pooled sTax peptide
P1 (sTax1-98)
P2 (sTax85-164)
P3 (sTax151-266)
P4 (sTax253-353)
IFN-γ spots / 10^5 cells
WT
rVV sTax M22
CD4-depleted
CD8-depleted
h
RT(-)
RT(+)
Non
WT -VV
rVV SBZ LL/AA
SBZ
β-actin
i
WT-VV (n=8)
rVV-SBZ LL/AA (n=9)
CD4-depleted
CD8-depleted
IFN-γ spots / 5x10^5 cells
j
Pooled SBZ peptide
P1 (SBZ1-62)
P2 (SBZ49-104)
P3 (SBZ91-152)
P4 (SBZ139-209)
IFN-γ spots / 10^5 cells
WT
rVV SBZ LL/AA
CD4-depleted
CD8-depleted

Figure S2 Generation of STLV-1 viral antigen-expressing vaccinia virus LC16m8 strain.

(a, b) Point mutations in HTLV-1 Tax M22 (**a**) and HBZ LL/AA (**b**) were inserted into the equivalent sites in sTax and SBZ. 293FT cells were transfected with HA-tagged WT or mutant plasmid. After 48 hours of transfection, expression level was determined by western blot using rabbit polyclonal anti-HA antibody. Full-length data is presented in Supplementary Figure 1a and b. Those constructs expressed mutant viral antigen at a similar level to WT constructs. (**c, d)** The effect of viral proteins on transcriptional activity was impaired by the point mutations in sTax and SBZ. (**c)** Vero cells were transfected with kB-Luc and sTax or sTax M22 and the luciferase activity was measured. (**d)** EL4 cells were transfected with WT-Luc, containing Tax-inducible CREs of HTLV-1 LTR, Tax and SBZ or SBZ LL/AA. The cells were harvested after 48 hours. Relative luciferase activities were calculated as the ratio of firefly to *Renilla* luciferase activities. The bars represent the mean ± SD of triplicate experiments. (**e-j)** rVV vaccination induces an antigen-specific T-cell response in mice. sTax (**e**) and SBZ (**h**) expression from the generated rVV strains were confirmed by Western blot or RT-PCR in RK13 cells after 48 hours of post-infection, respectively. Expression of sTax or HBZ mutants was detected using anti-Tax antibody (MI73) or SBZ primer set, respectively. Full-length data is presented in Supplementary Figure 1c and d. rVV vaccination and detection of the specific immune response in mice were performed as shown in (**f**). CD4- or CD8-depleted splenocytes were stimulated with pooled overlapped peptides of sTax (**f**) or SBZ (**i**) and T-cell responses were measured by ELISPOT. The total numbers of spots from the four pools (P1-P4) are shown. (**g, j**) The proportion of T-cell responses induced by each of the four pooled-peptides of sTax (**g**) and SBZ (**j**) is shown. A representative result is shown from at least two similar experiments. *, $P<0.05$ by t-test.

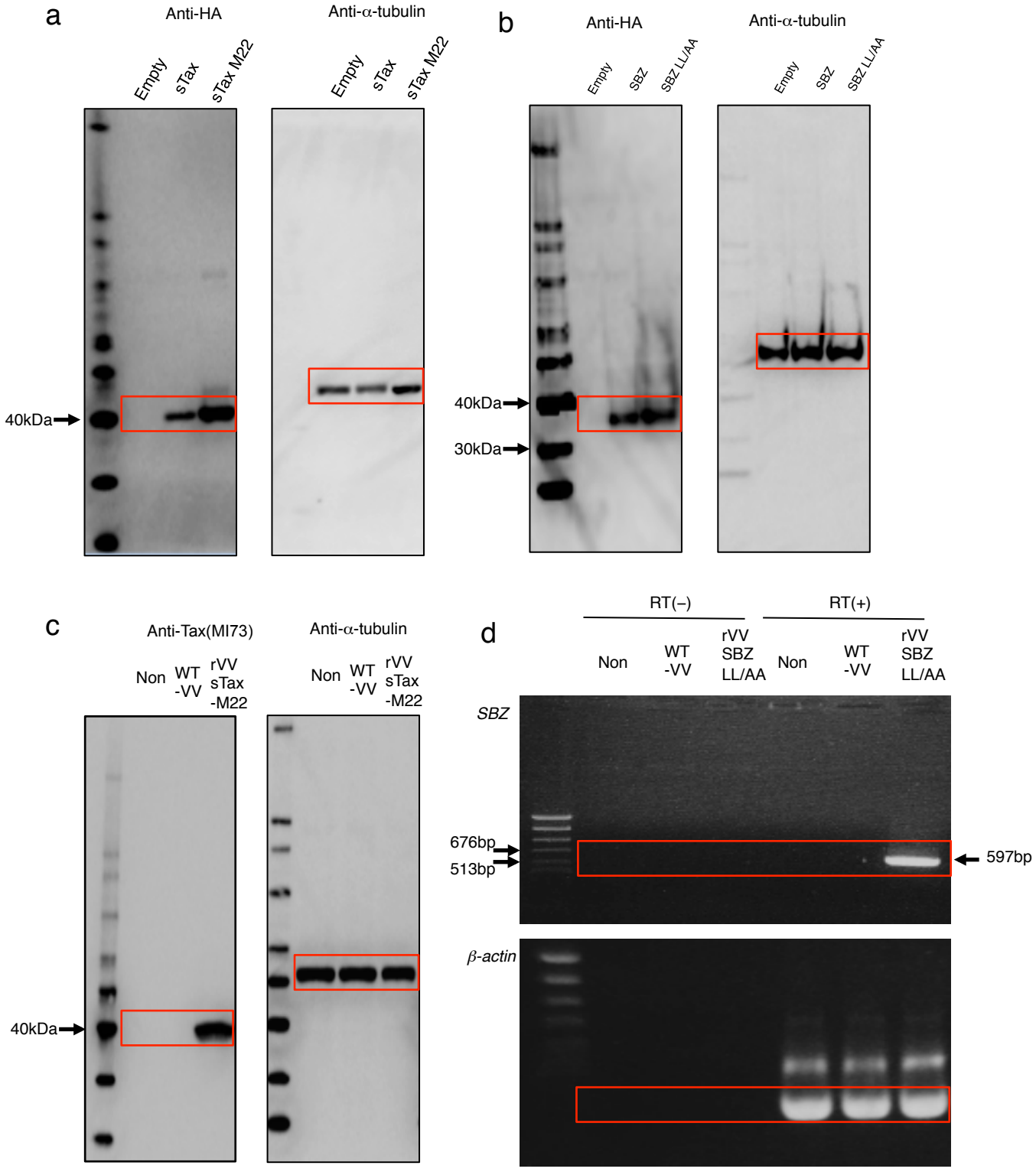

Figure S3 Full-length data of Figure S1

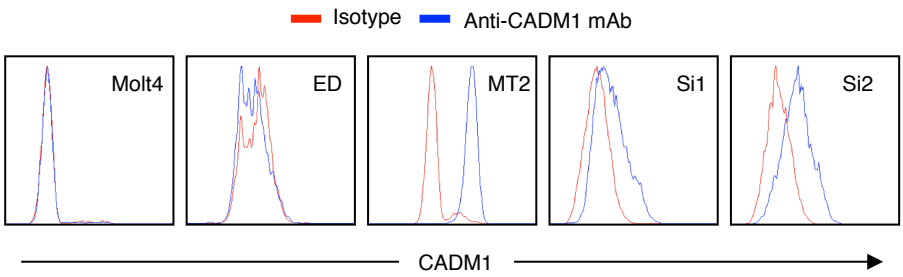

Figure S4 STLV-1 infected cells express CADM1.
We detected CADM1 expression using anti-human CADM1 mAb in cell lines. Si1 is a HTLV-1-immortalized monkey lymphocyte cell line. Si2 is a STLV-1-immortalized monkey lymphocyte cell line. Both T-cell lines derived from JMs express like a positive control, MT-2. ED and Molt4 are negative control for CADM1 expression.

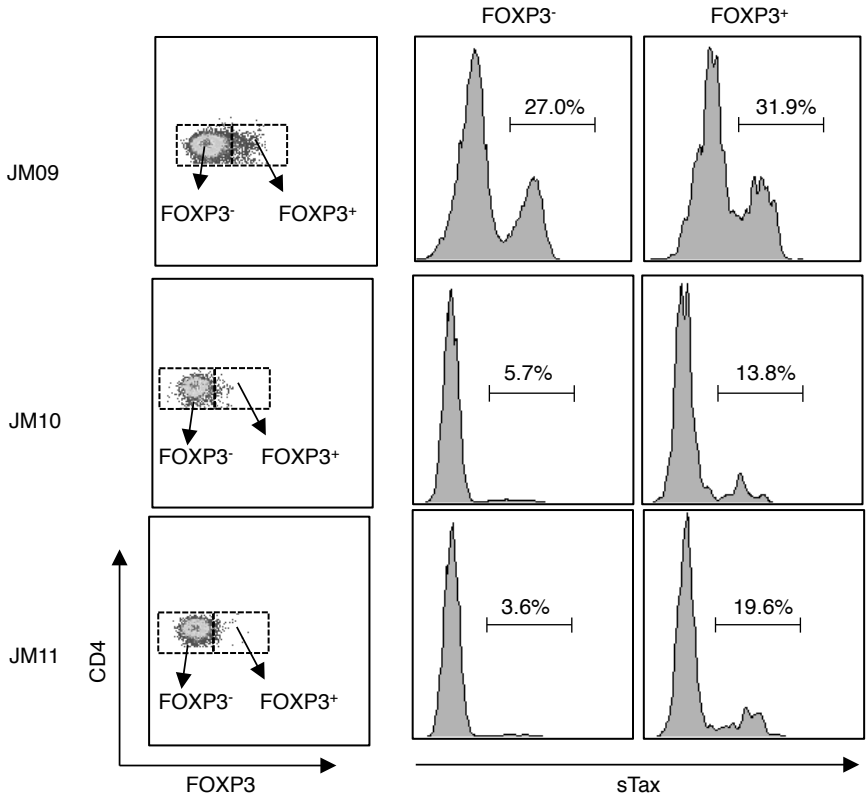

Figure S5 sTax expression in CD4+ FOXP3+ cells .
CD8-depleted monkey PBMCs were cultured for 24 hours. sTax expression was measured in CD4+ FOXP3- or CD4+ FOXP3+ cells. Positive cells were gated based on each isotype control (>0.2%).

Supplemental methods

Antibodies and reagents

The following antibodies were purchased from BD bioscience: purified mAbs to mouse IgG3 (R40-82) and CD28 (37.51) and human CD28 (28.2), CCR4 (1G1: recognizes a different epitope than mogamulizumab), IFN-g (4S.B3) and IL-2 (MQ1-17H12). MAbs to human CD4 (OKT-4), CD8a (RTP-T8), CD69 (FN50), and TNF-a (MAB11) were from Biolegend. MAbs to mouse CD11b (M1/70), human CD45RA (GRT22) and FOXP3 (PCH101) were from e-bioscience. Anti human CADM1 (3E1) was from MBL. Fluorescence-labeled anti-CCR4 antibody (clone KM2160, Kyowa Hakko Kirin Co. Ltd.), which is the original clone of mogamulizumab, was used for detection of $CCR4^+$ cells in monkey.

The following reagents were used for cell culture: protein transport inhibitor (BD PharMingen), streptavidin-PE (BD PharMingen), $OVA_{323-339}$(Bachem), tracer dye (CellTrace violet: Life Technologies) and human IL-2 (Wako). Mouse splenic T cells or monkey peripheral blood mononuclear cells (PBMCs) were depleted (<5%) or purified (>94%) using anti-mouse or anti-human CD4 or CD8 magnetic particles (BD Biosciences) according to the manufacturer's protocol. Whole overlap peptides (offset by 6 amino acids) were constructed based on the amino acid sequences of Tax, HBZ, sTax and SBZ (KURABO). All sequences of peptide used in this study are summarized in S1-S4 Table.

Cells

Suspension cell lines and primary cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and antibiotics. Si1 and Si2 cell lines were cultured in RPMI 1640 supplemented with 20% fetal bovine serum (FBS), antibiotics and IL-2. 2-Mercaptoethanol was added for culture of mouse cells. Vero, RK13 and chicken embryonic fibroblasts were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% newborn calf serum, antibiotics and tryptose phosphate broth. PBMCs were separated from human or monkey whole blood using Ficoll. Flow cytometry was carried out using a FACS Verse with Suite Software or FACS CantoII with FACSDiva software (BD Pharmingen). Data was analyzed by FlowJo software (Treestar).

Promoter assay

The PathDetect pNF-kB-Luc Cis-Reporter Plasmid was purchased from Promega. WT-Luc (containing Tax-inducible CREs from HTLV-1 LTR) was a gift from J. Fujisawa (Kansai medical university). sTax (WT, M22 (T130A and L131S)) and SBZ (WT and LL/AA (L27A and L28A)) constructs were cloned into pCAG-HA. Vero (10^5) or EL4 ($2x10^5$ cells) were transiently co-transfected 24 hours after cell seeding using Lipofectamine™ LTX (Invitrogen) with 300 ng of reporter plasmid, 30 ng of *Renilla* luciferase control vector (pRL-TK), and 600 ng of the expression plasmid. Transfected Vero and EL4 were harvested 48 hours after transfection, respectively. Firefly and *Renilla* luciferase activities were then measured using the Pickagene kit (Toyo Inki). Relative luciferase activities were calculated as the ratio of firefly to *Renilla* luciferase activities.