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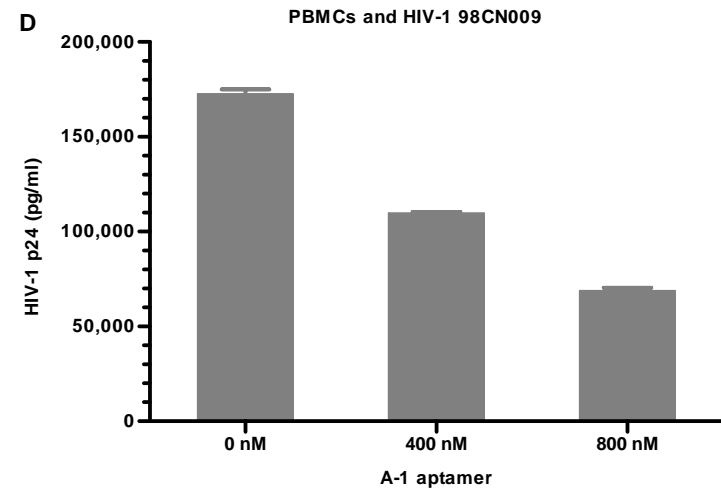
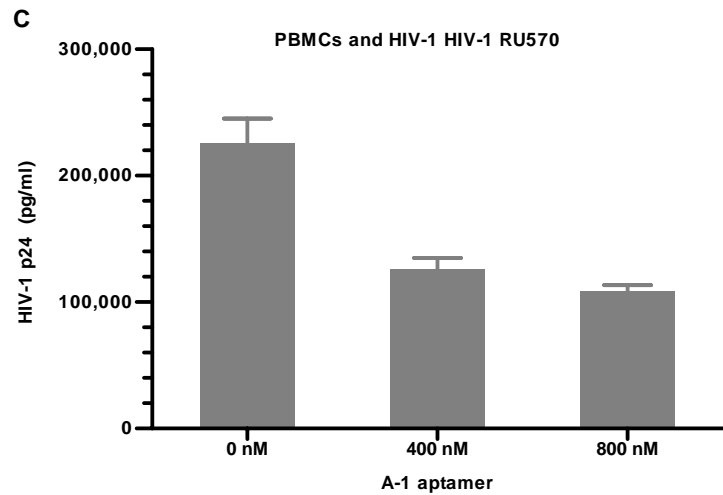
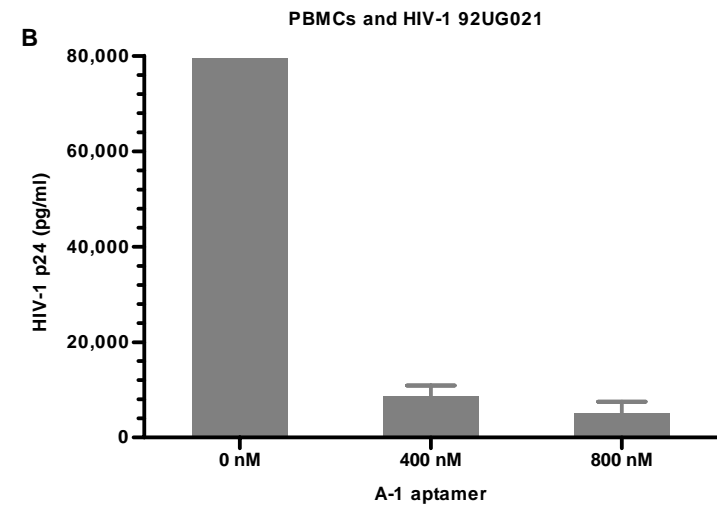
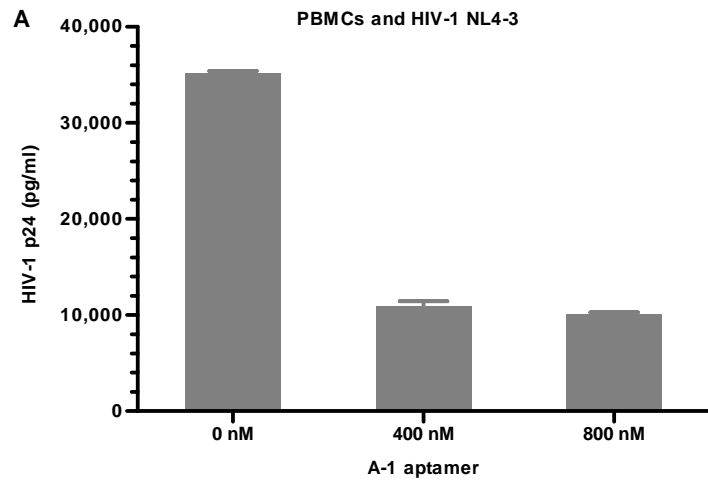
Table S1. HIV-1 viral loads in individual RAG-hu mice.

Table S2. CD4:CD3 T-cell ratios in treated and control individual RAG-hu mice.

Table S3. *P*-values for effects on HIV-1 viral loads in individual RAG-hu mice.

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Figure S1



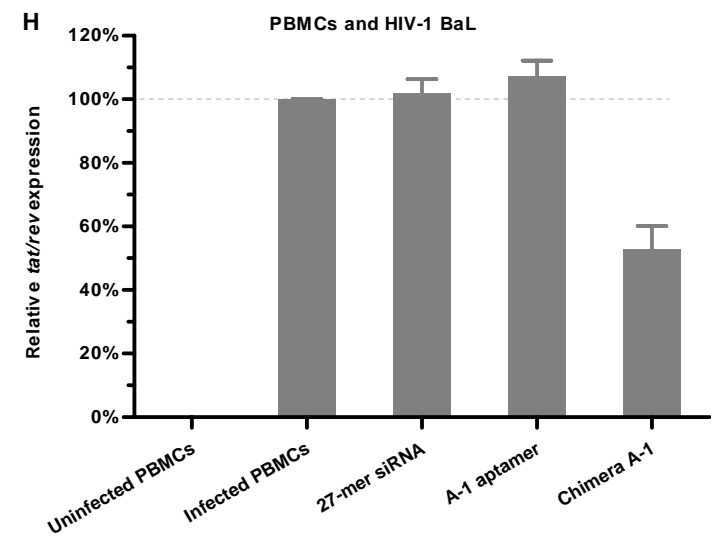
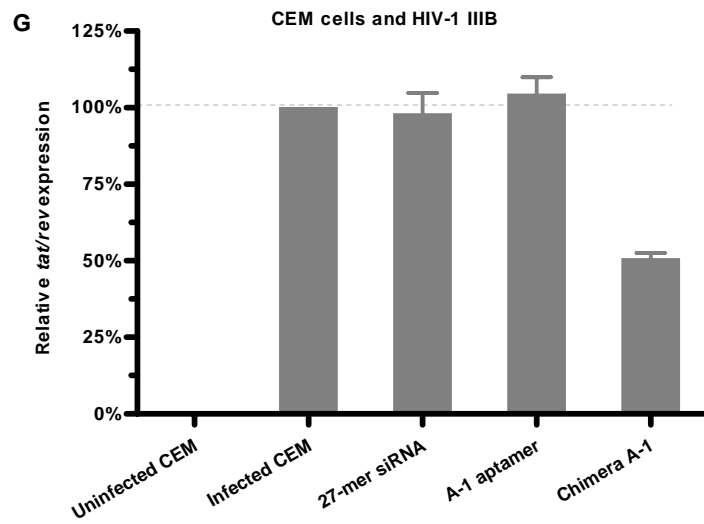
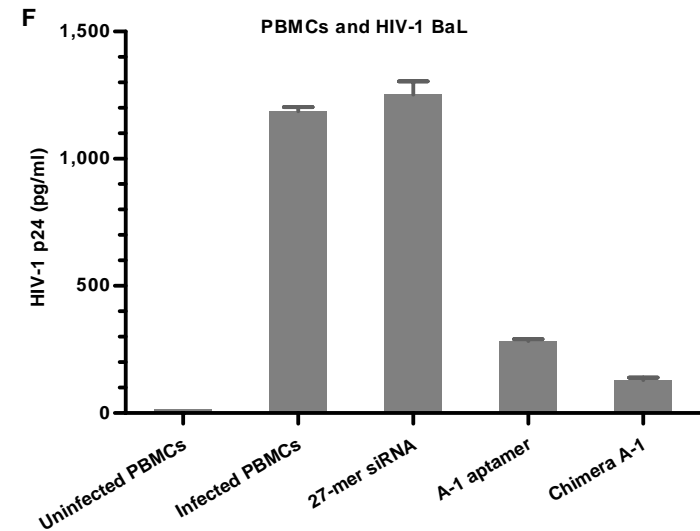
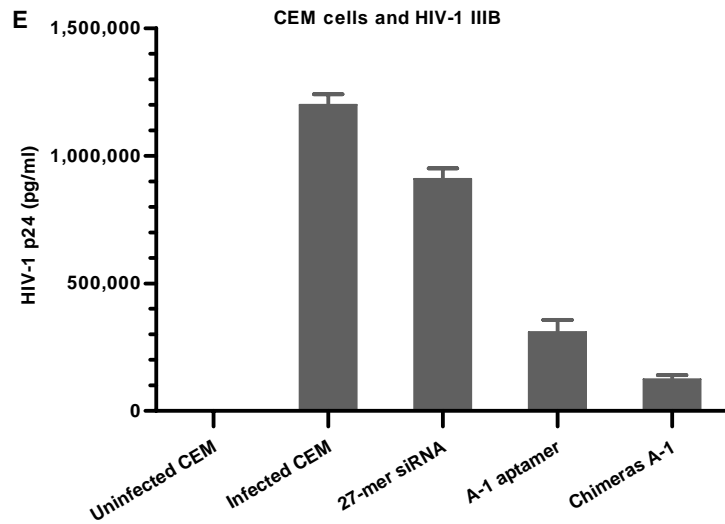


Fig. S1. Suppression of HIV-1 by A-1 aptamer and Ch A-1 chimera. (A-D) A-1 aptamer inhibition of HIV levels in PBMCs infected with various strains of HIV-1. Data are shown as concentration of HIV p24 core protein, measured 7 days after infection. (A) HIV-1 NL4.3, (B) HIV-1 92UG021, (C) HIV-1 RU570 and (D) HIV-1 98CN009. (E and F) A-1, Ch A-1, and siRNA inhibition of HIV-1 replication in (E) CEM cells infected with HIV-1 IIIB and in (F) PBMCs infected with HIV-1 BaL. Data are shown as concentration of HIV p24 core protein, measured 7 days after infection for (E) and 8 days after infection for (F). (G and H) Down regulation of *tat/rev* mRNA by A-1, Ch A-1, and siRNA in (G) HIV-1 IIIB-infected CEM cells and in (H) PBMCs cells infected with HIV-1 BaL. Data for *tat/rev* are shown relative to untreated, infected cells, measured 7 days after infection for (G) and 8 days after infection for (H). Error bars indicate SD ($N = 3$).

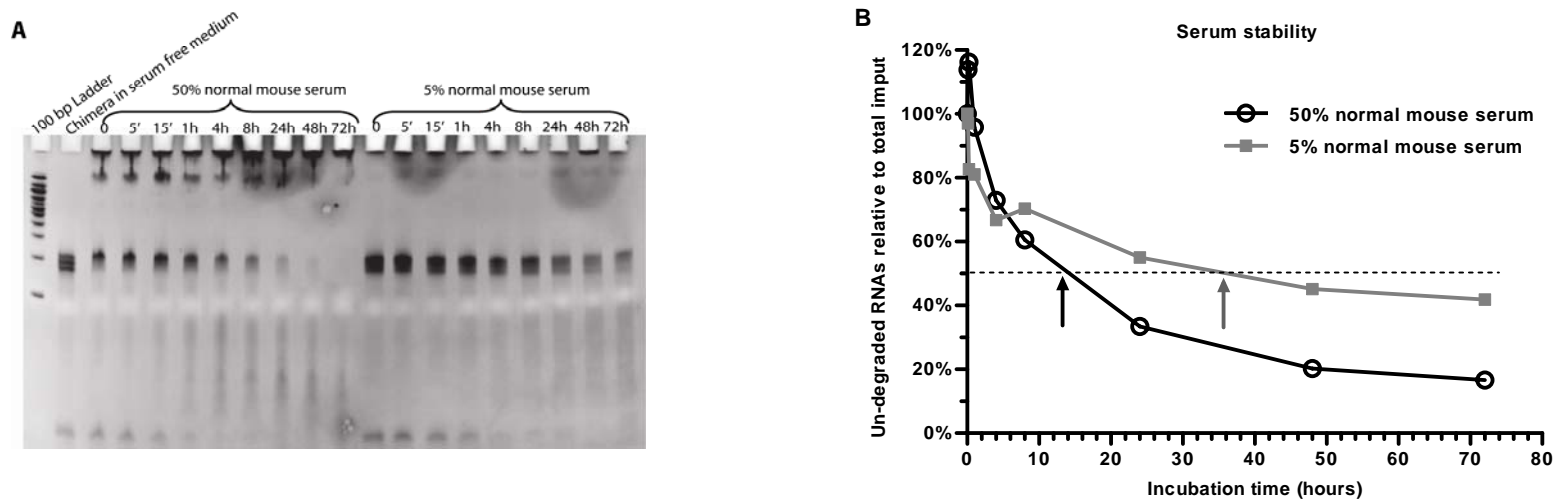


Fig. S2. Stability of 2'-fluoro modified Ch A-1 in mouse serum. (A) The Ch A-1 construct was incubated in 50% or 5% mouse serum (normal mouse serum, jackson immunoresearch laboratories) at 37°C for the lengths of time indicated and were resolved by 8% polyacrylamide electrophoresis. (B) The band intensities were quantitatively analyzed by Alphamager 3400 and percentage of un-degraded RNAs was calculated. As indicated by arrows, at 50% serum, 50% free RNA was retained at 16 h; at 5% serum, 50% free RNA was retained at 30 h.

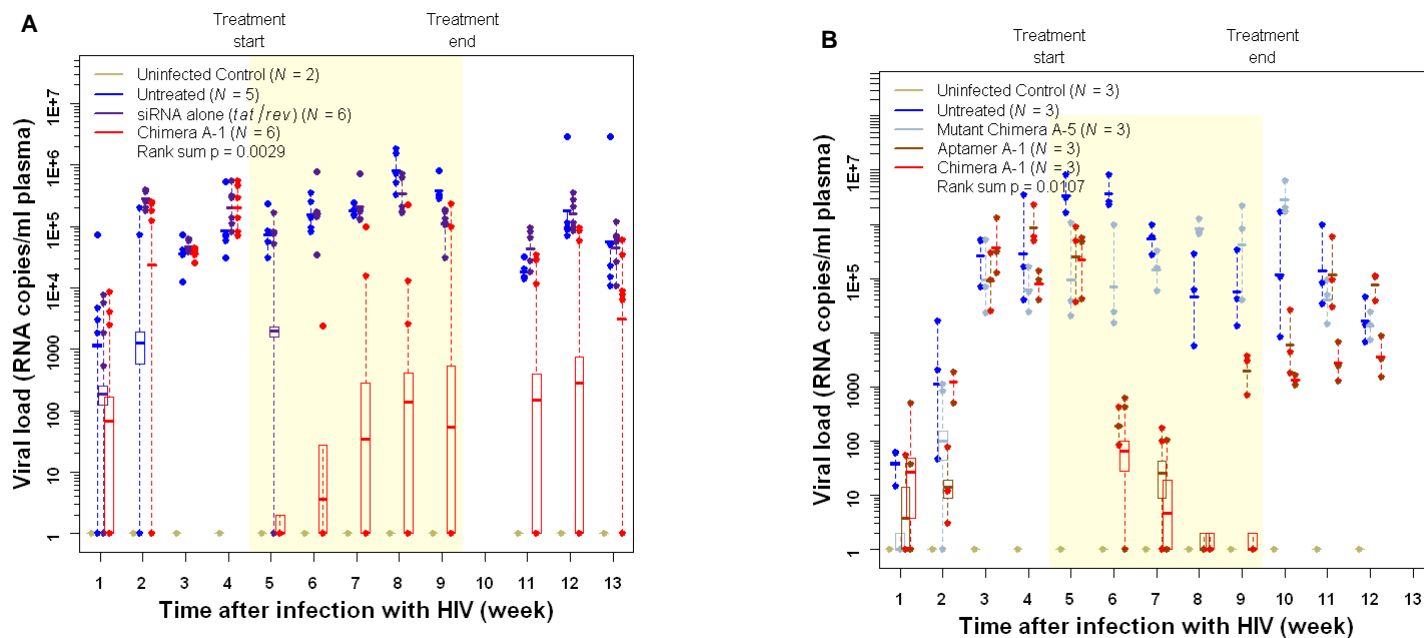


Fig. S3. Aptamer A-1 and Ch A-1 chimera, but not a mutant chimeric, suppress viral loads in HIV-1 infected RAG-hu mice. Mice were infected with HIV-1 at week 0, and 5 weeks later, weekly treatments were started. The treatment period is indicated by the yellow region. (A) Viral loads in uninfected mice ($N = 2$), untreated mice ($N = 5$), *tat/rev* siRNA only treated mice ($N = 6$) and Ch A-1--treated mice ($N = 6$). (B) In a separate experiment, viral loads in uninfected mice, ($N=3$), non-treated mice ($N=3$), A-1 aptamer--treated mice ($N=3$), Ch A1 chimera--treated mice ($N=3$), and mutant A-5 chimera--treated mice ($N=3$). The viral RNA was detected through qRT-PCR as described in Methods. Thus, if there was no detectable viral RNA we established this as a value of 1 (100) to allow for the use of logarithmic values on the Y-axis. P values for the effects of A-1 and Ch A-1 are indicated and were calculated as described in Materials and Methods. Box plot boundaries are ± 1 SD around the mean (horizontal line). Points have been jittered for easier interpretation.

Figure S4

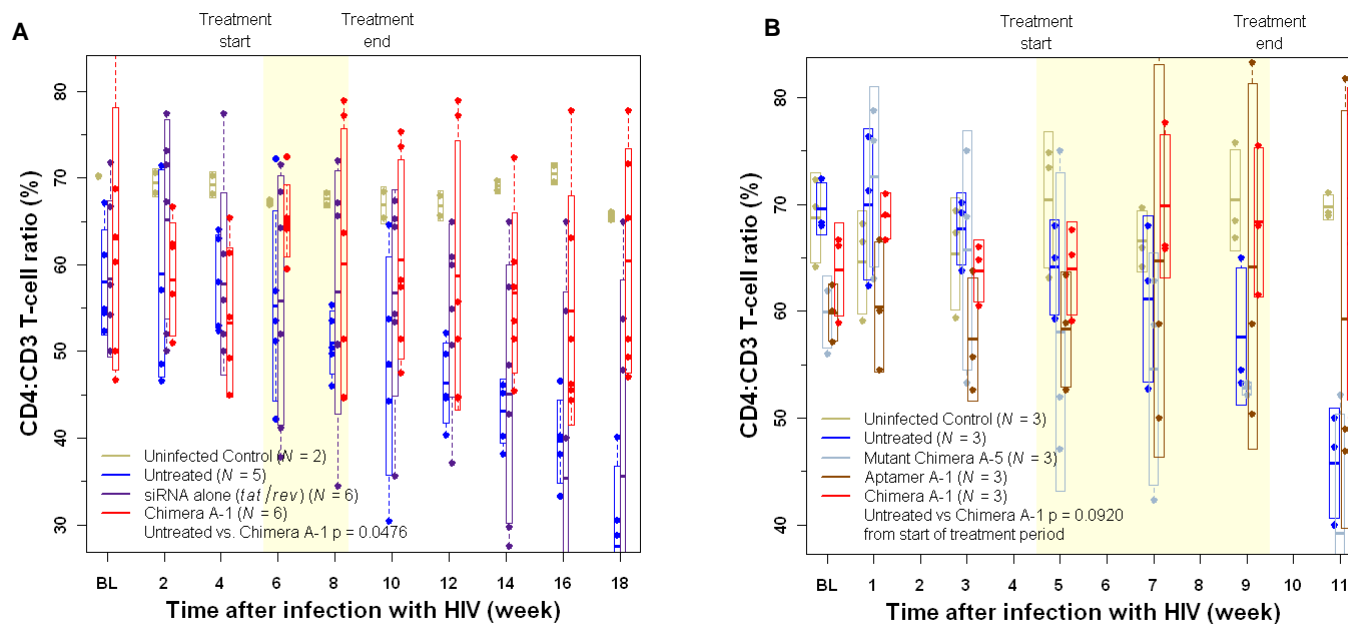


Fig. S4. Protection of RAG-hu mice from CD4 T-cell loss by the Ch A-1 chimera. CD4 T-cell levels were assessed by FACS at each indicated week before and after siRNA treatment. “BL” refers to the baseline measurement. Treatment duration is indicated by the yellow region. (A) Mice from the first experiment (shown in Fig. 2A) group. Uninfected mice ($N = 2$), untreated mice ($N = 5$), *tat/rev* siRNA treated mice ($N = 6$), chimera Ch A-1 treated mice ($N = 6$). (B) Mice from second experiment (shown in Figure 2B). CD4:CD3 T-cell ratios were assessed by FACS at each indicated week before and after treatment. Treatment duration and experimental animals are as in (A). Box plot boundaries are ± 1 SD around the mean (horizontal line). Points have been jittered for easier interpretation. P values for both experiments were determined as described in Materials and Methods. BL (base line): each individual mouse was bled two times prior to HIV-1 infection and averaged within treatment groups to establish a baseline CD4:CD3 level.

Figure S5

Tat/rev site I domain: 5982-6008

HIV-1 gp120 domain: 6320...7747 nt

HIV-1 gp41 domain: 7796...8782 nt

Index of nucleotides	Reference	Allele	SNP %	Coverage	Overlapping annotations	Amino_Acid_Change
5990	A	A/G	0.186	71,352	CDS: tat protein, CDS: rev protein	Gln -> Arg; Ser -> Gly
5991	G	G/T	0.158	69,764	CDS: tat protein, CDS: rev protein	Gln -> His; Ser -> Ile
5994	A	A/C	0.161	64,744	CDS: tat protein, CDS: rev protein	Asp -> Ala
5996	G	G/A	0.273	65,305	CDS: tat protein, CDS: rev protein	Glu -> Lys; Arg -> Gln
6002	C	C/A	0.441	64,814	CDS: tat protein, CDS: rev protein	Ala -> Asp; Leu -> Ile
6005	A	A/T	0.173	65,486	CDS: tat protein, CDS: rev protein	His -> Leu; Ile -> Phe
6007	C	C/A	0.258	65,425	CDS: tat protein, CDS: rev protein	Gln -> Lys
Index of nucleotides	Reference	Allele	SNP %	Coverage	Overlapping annotations	Amino_Acid_Change
6486	T	T/G	1.035	24444	CDS: envelope polyprotein	Val -> Gly
6523	A	A/T	8.664	30966	CDS: envelope polyprotein	
6559	T	T/A	1.231	31687	CDS: envelope polyprotein	Asp -> Glu
6672	A	A/G	1.16	25090	CDS: envelope polyprotein	Lys -> Arg
6743	T	T/A	1.599	26580	CDS: envelope polyprotein	Phe -> Ile
6752	A	A/G	5.323	27070	CDS: envelope polyprotein	Lys -> Glu
6779	A	A/G	1.427	22843	CDS: envelope polyprotein	Thr -> Ala
6800	T	T/C	1.288	27785	CDS: envelope polyprotein	Cys -> Arg
6924	G	G/T	3.19	28590	CDS: envelope polyprotein	Gly -> Val
6943	C	C/T	1.359	30615	CDS: envelope polyprotein	
6999	A	A/G	1.234	24955	CDS: envelope polyprotein	Asn -> Ser
7016	G	G/A	1.237	22071	CDS: envelope polyprotein	Glu -> Lys
7113	A	A/G	1.427	22361	CDS: envelope polyprotein	Asn -> Ser
7137	G	G/A	2.029	19907	CDS: envelope polyprotein	Arg -> His
7169	A	A/G	1.758	16101	CDS: envelope polyprotein	Thr -> Ala
7213	T	T/C	1.008	15083	CDS: envelope polyprotein	
7224	A	A/G	1.293	15236	CDS: envelope polyprotein	Lys -> Arg
7232	G	G/A	1.166	15271	CDS: envelope polyprotein	Ala -> Thr
7332	C	C/T	1.02	18037	CDS: envelope polyprotein	Thr -> Met
7384	G	G/A	14.452	21464	CDS: envelope polyprotein	
7396	T	T/C	1.421	18721	CDS: envelope polyprotein	
7399	G	G/A	13.351	18725	CDS: envelope polyprotein	Trp -> Stp
7419	C	C/T	2.662	19419	CDS: envelope polyprotein	Thr -> Ile
7424	G	G/A	1.119	19834	CDS: envelope polyprotein	Gly -> Arg
7433	A	A/C	3.382	19397	CDS: envelope polyprotein	Asn -> His
7452	C	C/T	2.653	17907	CDS: envelope polyprotein	Thr -> Ile
7455	T	T/C	1.018	20226	CDS: envelope polyprotein	Ile -> Thr
7477	A	A/G	1.406	20632	CDS: envelope polyprotein	
7505	G	G/A	3.397	25404	CDS: envelope polyprotein	Gly -> Arg
7590	G	G/A	1.186	20568	CDS: envelope polyprotein	Gly -> Asp
7649	T	T/C	1.06	28478	CDS: envelope polyprotein	Trp -> Arg
7677	T	T/C	1.708	30621	CDS: envelope polyprotein	Val -> Ala
7875	A	A/C	2.897	65831	CDS: envelope polyprotein	Asn -> Ser
8014	A	A/G	4.206	55260	CDS: envelope polyprotein	
8048	G	G/A	4.537	55258	CDS: envelope polyprotein	Ala -> Thr
8292	T	T/C	1.641	21272	CDS: envelope polyprotein	Val -> Ala

Fig. S5. Sequence analysis of mutant virus from animals treated with Ch A-1. Viral RNAs were isolated from plasma of Ch A-1 treated mice and sequence changes in *tat/rev*, *gp120* and *gp41* sequences were analyzed. Positions of mutations unique to the Ch A-1 treated animals are listed. *gp120*, pink; *gp41*, blue; *tat/rev*, yellow.

Table S1

Table S1. HIV-1 viral loads in individual RAG-hu mice. (A) Individual and average viral loads are indicated for the experiment described in Fig. 2A. (B) Individual and average viral loads are indicated for the experiment described in Fig. 2B. Nondetectable samples were set to a value of 1.

A

Viral Loads (copies per ml plasma)

		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8	Week 9	Week 11	Week 12	Week 13
Uninfected control	J210	1	1	1	1	1	1	1	1	1	1	1	1
	J209	1	1	1	1	1	1	1	1	1	1	1	1
	Average	1	1	1	1	1	1	1	1	1	1	1	1
Untreated	J258	3033	204054	41166	57616	55400	347675	174354	713219	284795	15088	113488	49082
	J257	4619	1	41166	66418	56345	254748	150336	517821	305474	14198	94170	23024
	J256	1857	204054	34886	30386	31203	81200	185884	1537897	814993	31291	69000	10800
	J246	73500	73500	73500	73500	84300	94170	153396	330765	327654	14198	88491	15235
	J245	1	1	12233	535000	234000	136769	240146	1863603	327654	20447	2881351	
	Average	16602	96322	40590	152584	92250	182912	180823	992661	412114	19044	649300	24535
siRNA (<i>tat/rev</i>) alone	J273	526	254606	41166	295553	1	164826	126587	163540	114497	93455	271097	120074
	J266	5700	204054	37896	317328	167358	154886	186000	627489	106746	82757	347675	56325
	J264	1	368192	41166	560401	76566	780432	134958	211280	131728	18106	211385	69240
	J253	1	368192	62268	82207	82200	175403	713219	627489	31200	64894	113488	26421
	J217	1857	396383	57322	109245	51400	145546	185884	174354	174359	27709	94170	64635
	J216	7545	176061	41166	135215	1	34767	174354	713219	187019	27709	83155	10800
	Average	2605	294581	46831	249992	62921	294795	253500	419562	124258	52438	186828	57916
Chimera A-1	J276	4015	121747	37896	71312	1	1	15877	13102	230786	34793	57255	60337
	J274	8681	176061	34886	82207	1	1	1	1	1	1	1	7656
	J272	2458	1	44718	452770	1	1	1	1	Dead			
	J251	1	236498	34886	135215	1	1	1	2566	1	28303	94170	6228
	J249	1	254606	25053	295553	1	1	1	1	1	1	1	8785
	J247	1	121747	41166	560401	1	2368	97984	225251	99520	11569	83155	34793
	Average	2526	151777	36434	266243	1	396	18978	45564	24881	14934	46916	23560

Table S1

Table S1. HIV-1 viral loads in individual RAG-hu mice. (A) Individual and average viral loads are indicated for the experiment described in Fig. 2A. (B) Individual and average viral loads are indicated for the experiment described in Fig. 2B. Nondetectable samples were set to a value of 1.

B

Viral Loads (copies per ml plasma)

		Week1	Week2	Week3	Week4	Week5	Week6	Week7	Week8	Week9	Week10	Week11	Week12
Uninfected Control	J570	1	1	1	1	1	1	1	1	1	1	1	1
	J571	1	1	1	1	1	1	1	1	1	1	1	1
	J572	1	1	1	1	1	1	1	1	1	1	1	1
	Average	1	1	1	1	1	1	1	1	1	1	1	1
Untreated	J565	15	46	70653	40173	1611916	2655213	978487	60589	13505	107329	81927	13975
	J566	64	16469	526913	167200	3062118	8307855	270938	291214	335899	1731311	988012	46677
	J567	60	2016	491653	3579558	8307830	2302355	593930	5652	42427	8190	33857	6770
	Average	46	6177	363073	1262310	4327288	4421808	614452	119152	130610	615610	367932	22474
Mutant chimera A-5	J554	1	1146	23277	56175	39446	24263	320608	716431	841330	2035506	49469	24459
	J555	1	849	70653	24425	20593	14871	59073	1257084	39690	6266084	14665	7238
	J556	1	1	526913	166751	1070324	987725	155435	661132	2205710	1733448	93791	13743
	Average	1	665	206948	82450	376788	342286	178372	878216	1028910	3345013	52641	15147
Aptamer A-1	J547	1	3	93229	599425	911450	83	176	1	709	26559	93791	113621
	J548	1	12	302753	494757	494491	188	1	1	2961	4489	29650	38287
	J549	56	79	24952	2296677	36377	429	98	1	3791	1820	599471	106580
	Average	19	31	140312	1130286	480773	233	92	1	2487	10956	240971	86162
Chimera A-1	J456	1	1921	308042	137626	562806	628	104	1	1	1102	2435	3346
	J458	37	1921	125161	40783	479243	429	1	1	1	1304	6800	8774
	J460	510	495	1319090	93745	42772	1	1	1	1	1675	1278	1539
	Average	183	1445	584097	90718	361607	353	35	1	1	1360	3504	4553

Table S2

Table S2. CD4:CD3 T-cell ratios in treated and control individual RAG-hu mice. CD4:CD3 T-cell ratios were determined by FACS analysis. Cell levels are indicated relative to the baseline established prior to infection. Values are shown for individual mice. (A) Experiment in Figure 5A. (B) Experiment in Figure 5B.

A CD4:CD3 T-cell ratio

Uninfected Control

	Initial	Week2	Week4	Week6	Week8	Week10	Week12	Week14	Week16	Week18
J209	70.2	70.6	70.3	66.9	66.9	65.4	68	69.7	71.4	65.2
J210	70.3	68.3	68.2	67.5	68.3	68.4	65.6	68.5	69.6	66.1
Average	70.25	69.45	69.25	67.2	67.6	66.9	66.8	69.1	70.5	65.65
to baseline	1	0.988612	0.985765	0.956584	0.962278	0.952313	0.95089	0.98363	1.003559	0.93452

Untreated

	Initial	Week2	Week4	Week6	Week8	Week10	Week12	Week14	Week16	Week18
J258	67.2	57.1	63	72.3	55.4	53.7	44.8	38.1		28.8
J257	54.5	48.5	58	42.2	46	44.3	44.6	45.2	46.6	30.5
J256	52.4	71.4	53	51.2	49.7	48.5	49.7	46.1	33.3	23.1
J246	54.9	46.6	52.4	57	50.4	30.4	40.4	40.2		15
J245	61.1	71.4	64	53.5		64.6	52.1	46.1	39.9	40.1
Average	58.02	59	58.08	55.24	50.375	48.3	46.32	43.14	39.93333	27.5
to baseline	1	1.016891	1.001034	0.952085	0.868235	0.832472	0.798345	0.743537	0.688268	0.473974

siRNA (*tat/rev*) alone

	Initial	Week2	Week4	Week6	Week8	Week10	Week12	Week14	Week16	Week18
J273	57.7	73.2	56	64.3	67.2	64.4	60.9	57.4	54.7	53.8
J266	66.7	71.5	50	71.6	50.9	53.4	50.7	48.4	40	47.9
J264	71.8	67.3	61.3	68.4	50.7	54.3	54.9	29.7	13.3	12.3
J253	50	77.4		37.8	72	65.3	60	42.8	24.9	14.4
J217	54.2	52			65.7	67.4	65			
J216	50			41.2	34.5	35.6	37.1	27.5	14.6	20
Average	58.4	68.28	55.76667	56.66	56.83333	56.73333	54.76667	41.16	29.5	29.68
to baseline	1	1.169178	0.954909	0.970205	0.973174	0.971461	0.937785	0.704795	0.505137	0.508219

Chimera A-1

	Initial	Week2	Week4	Week6	Week8	Week10	Week12	Week14	Week16	Week18
J276	46.7	56.7	49.3	64.2	44.7	58.3	44.7	45.4	45.5	47.1
J274	63.2	62.1	45	64.2	44.6	47.5	44.6	53.5	44.4	49.4
J272	68.8	51	54	64.6	51.4					
J251	50	51	45	59.5	63.7	57.4	55.7	60.3	77.8	
J249	60.3	66.7	61.4	65.4	77.2	73.6	79	57.5	46.2	65.4
J247	88.9	62.4	65.4	72.5	79	75.4	77.2	72.4	63.1	71.7
Average	62.98333	58.31667	53.35	65.06667	60.1	62.44	60.24	57.82	55.4	58.4
to baseline	1	0.925906	0.847049	1.033078	0.954221	0.991373	0.956444	0.918021	0.879598	0.927229

Table S2

Table S2. CD4:CD3 T-cell ratios in treated and control individual RAG-hu mice. CD4:CD3 T-cell ratios were determined by FACS analysis. Cell levels are indicated relative to the baseline established prior to infection. Values are shown for individual mice. (A) Experiment in Figure 5A. (B) Experiment in Figure 5B.

B

CD4:CD3 T-cell ratio

Uninfected Control	Initial	Week1	Week3	Week5	Week7	Week9	Week11
J571	72.3	68.2	69.4	74.8	65.9	68.5	71.1
J572	64.1	59.1	59.4	63.1	64.11	66.9	69.2
J573	69.8	66.5	67.3	73.4	69.7	75.8	68.9
Average	68.73333	64.6	65.36667	70.43333	66.57	70.4	69.73333
to baseline	1	0.939864	0.951018	1.024733	0.968526	1.024248	1.014549

Untreated	Initial	Week1	Week3	Week5	Week7	Week9	Week11
J565	68.3	76.3	69.2	65	62.8	54.5	40
J566	72.4	71.3	70.1	68		65	47.3
J567	68	62.4	63.8	59.3	52.7	53.3	50
Average	69.56667	70	67.7	64.1	57.75	57.6	45.76667
to baseline	1	1.006229	0.973167	0.921418	0.830139	0.827983	0.657882

Mutant chimera A-5	Initial	Week1	Week3	Week5	Week7	Week9	Week11
J554	61.9	78.8	75		42.3	53.3	33.3
J555	56	63	53.3	47.1	58.7	52.9	32.3
J556	61.9	76	68.8	52	62.8	52.1	
Average	59.93333	72.6	65.7	49.55	54.6	52.76667	32.8
to baseline	1	1.211346	1.096218	0.826752	0.911012	0.880423	0.547275

Aptamer A-1	Initial	Week1	Week3	Week5	Week7	Week9	Week11
J549	62.5	54.5	55.7	58.9	85.3	83.3	81.8
J548	57.1	60	52.6		50	50.4	46.9
J547	60	66.7	63.8	63.4	58.8		49
Average	59.86667	60.4	57.36667	61.15	64.7	66.85	59.23333
to baseline	1	1.008909	0.958241	1.021437	1.080735	1.116648	0.989421

Chimera A-1	Initial	Week1	Week3	Week5	Week7	Week9	Week11
J456	66.7	69	60.5	67.6	66.1	61.5	50
J458	66.1	71	66	59.1	65.8	68	78.4
J460	58.9	66.7	64.8	65.3	77.6	75.5	70.5
Average	63.9	68.9	63.76667	64	69.83333	68.33333	66.3
to baseline	1	1.078247	0.997913	1.001565	1.092853	1.069379	1.037559

Table S3

Table S3. *P* values of HIV-1 viral loads in individual RAG-hu mice. (A) Individual *P* value comparisons are calculated for the experiment described in Fig. 2A. (B) Individual *P* value comparisons are calculated for the experiment described in Fig. 2B.

A: Statistical analysis of viral load data

Comparison			<i>P</i> value
Uninfected Control	vs	Untreated	<i>P</i> = 0.0476
Uninfected Control	vs	siRNA (<i>tat/rev</i>) alone	<i>P</i> = 0.0357
Uninfected Control	vs	Chimera A-1	<i>P</i> = 0.0714
Untreated	vs	siRNA (<i>tat/rev</i>) alone	<i>P</i> = 1.0000
Untreated	vs	Chimera A-1	<i>P</i> = 0.0065
siRNA (<i>tat/rev</i>) alone	vs	Chimera A-1	<i>P</i> = 0.0022

Overall *P* value = 0.0029

B: Statistical analysis of viral load data

Comparison			<i>P</i> value
Uninfected Control	vs	Untreated	<i>P</i> = 0.0024
Uninfected Control	vs	Mutant chimera A-5	<i>P</i> = 0.0007
Uninfected Control	vs	Aptamer A-1	<i>P</i> = 0.0009
Uninfected Control	vs	Chimera A-1	<i>P</i> = 0.0023
Untreated	vs	Mutant chimera A-5	<i>P</i> = 0.2900
Untreated	vs	Aptamer A-1	<i>P</i> = 0.0106
Untreated	vs	Chimera A-1	<i>P</i> = 0.0036
Mutant chimera A-5	vs	Aptamer A-1	<i>P</i> = 0.0010
Mutant chimera A-5	vs	Chimera A-1	<i>P</i> = 0.0005
Aptamer A-1	vs	Chimera A-1	<i>P</i> = 0.0418

Overall *P* value = 0.0107

Table S4A:

Table S4. *P* values of CD4 T-cell levels in treated and control individual RAG-hu mice. (A) Individual *P* value comparisons are calculated for the experiment described in Fig. 2A. (B) Individual *P* value comparisons are calculated for the experiment described in Fig. 2B.

A: Statistical analysis of CD4:CD3 T-cell data

Comparison			<i>P</i> value
Uninfected Control	vs	Untreated	<i>P</i> = 0.0476
Uninfected Control	vs	siRNA (<i>tat/rev</i>) alone	<i>P</i> = 0.0714
Uninfected Control	vs	Chimera A-1	<i>P</i> = 0.2500
Untreated	vs	siRNA (<i>tat/rev</i>) alone	<i>P</i> = 0.4307
Untreated	vs	Chimera A-1	<i>P</i> = 0.0476
siRNA (<i>tat/rev</i>) alone	vs	Chimera A-1	<i>P</i> = 0.2944

Overall *P* value = 0.0736

B: Statistical analysis of CD4:CD3 T-cell data

Comparison			<i>P</i> value
Uninfected Control	vs	Untreated	<i>P</i> = 0.1872
Uninfected Control	vs	Mutant chimera A-5	<i>P</i> = 0.0577
Uninfected Control	vs	Aptamer A-1	<i>P</i> = 0.2830
Uninfected Control	vs	Chimera A-1	<i>P</i> = 0.7031
Untreated	vs	Mutant chimera A-5	<i>P</i> = 0.3284
Untreated	vs	Aptamer A-1	<i>P</i> = 0.7860
Untreated	vs	Chimera A-1	<i>P</i> = 0.2518
Mutant chimera A-5	vs	Aptamer A-1	<i>P</i> = 0.6726
Mutant chimera A-5	vs	Chimera A-1	<i>P</i> = 0.0742
Aptamer A-1	vs	Chimera A-1	<i>P</i> = 0.3512

Overall *P* value = 0.1147