

Novel *ex vivo* approaches distinguish effective and ineffective single agents for reversing HIV-1 latency *in vivo*

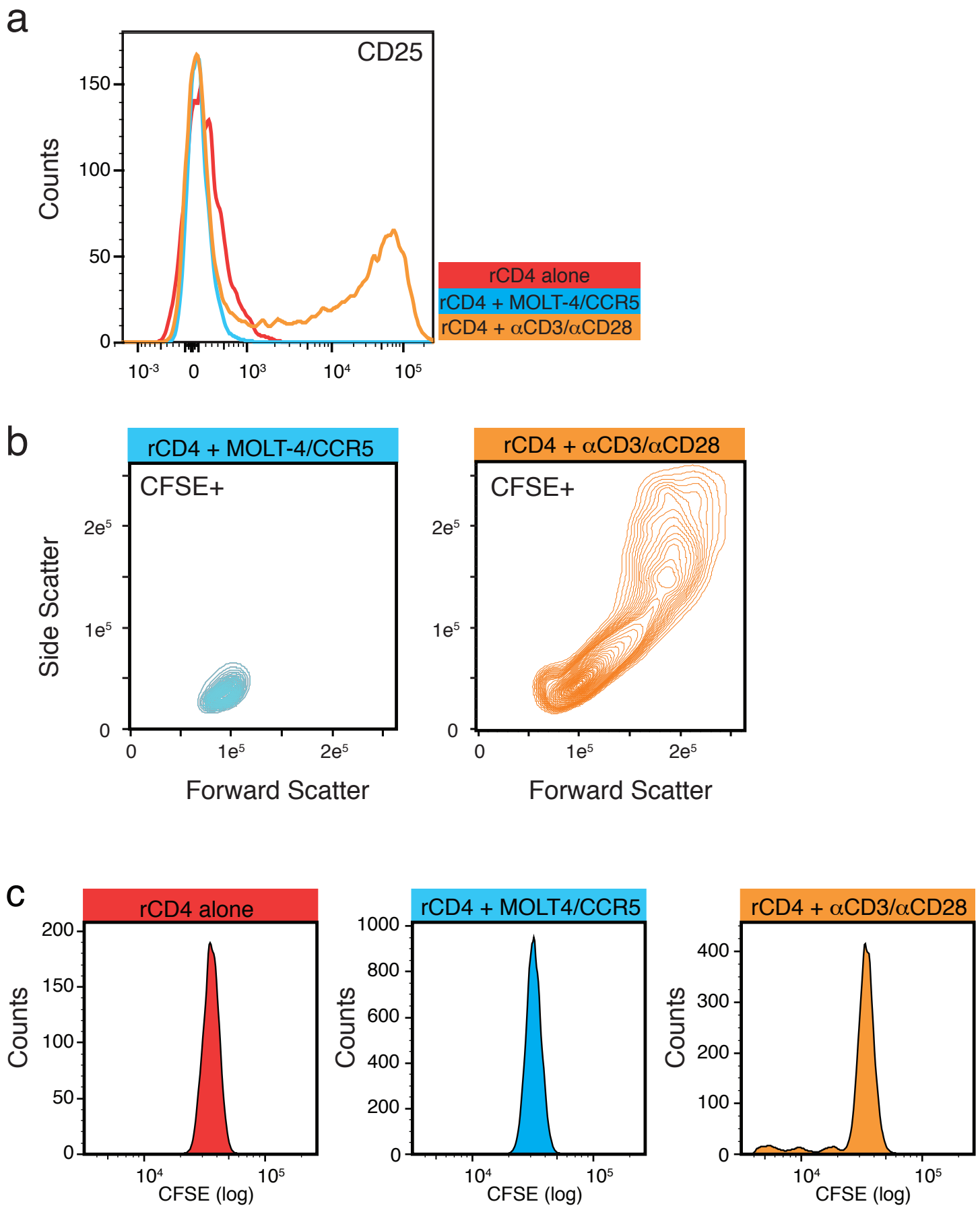
C. Korin Bullen†, Gregory M. Laird†, Christine M. Durand, Janet D. Siliciano, Robert F. Siliciano*

†These authors contributed equally to this work

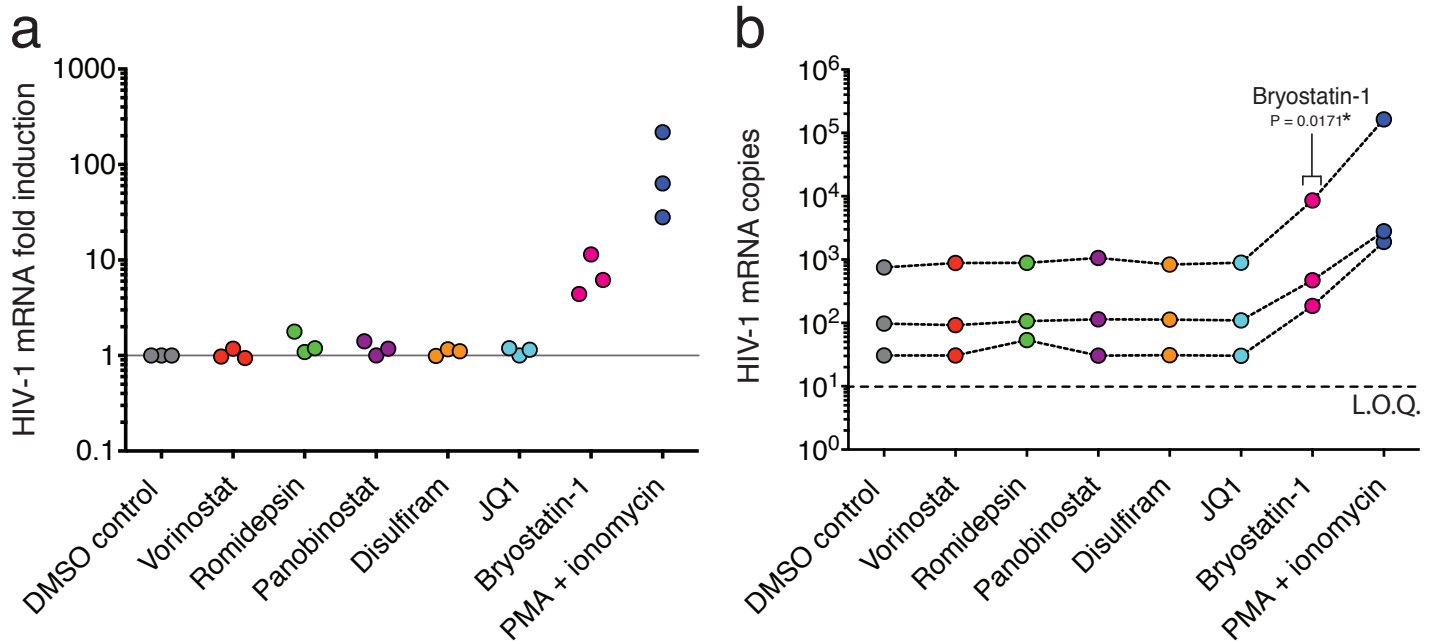
Supplementary Table 1. Characteristics of HIV-1 infected study participants.

Pt. ID	Age	Sex	Race	Duration of infection (months)	ART regimen	Time on ART (months)	Time on Suppressive ART (months)	Peak reported viral load (copies mL ⁻¹)
S1	53	M	W	307	DRV/r, RAL	152	20	710,579
S2	48	M	W	229	ABC, FTC, FPV/r	226	103	30,000
S3	78	M	W	101	FTC, TDF, EFV	68	57	750,000
S4	31	M	W	100	FTC, TDF, EFV	78	65	74,934
S5	52	M	W	208	ETR, DRV/r	200	120	750,000
S6	47	M	W	50	FTC, TDF, ATV/r	38	31	64,137
S7	52	M	B	216	EFV, DRV/r, RAL	192	16	—
S8	57	M	B	88	FTC, TDF, EFV	87	54	100,000
S9	53	M	B	153	FTC, TDF, LPV/r	144	66	69,956
S10	67	M	W	220	ABC, 3TC, EFV	202	92	—
S11	49	M	B	143	FTC, TDF, EFV	120	96	—
S12	36	M	B	60	FTC, TDF, NVP	60	60	—
S13	60	M	B	143	FTC, TDF, EFV	142	132	—
S14	49	M	B	194	FTC, TDF, RPV	92	20	—
S15	51	M	B	264	FTC, TDF, RAL	128	18	—
S16	52	M	B	235	FTC, TDF, EFV	192	80	—
S17	55	F	B	296	3TC, ETR, RAL	202	108	68,712
S18	50	M	B	103	3TC, TDF, DRV/r	>36	18	27,817
S19	38	F	B	139	DRV/r, RAL, MVC	48	24	—
S20	47	M	B	234	3TC, TDF, EVG/c	216	24	100,000
S21	45	M	W	79	FTC, TDF, EFV	72	60	60,000
S22	55	F	B	66	ABC, FTC, EFV	70	64	39,305
S23	54	F	B	180	3TC, TDF, EVG/c	96	9	—
S24	43	M	B	44	FTC, TDF, EFV	>12	12	—
S25	82	M	W	230	FTC, TDF, EFV	>84	84	—
S26	45	M	W	83	FTC, TDF, EFV	82	80	60,700
S27	41	M	B	228	FTC, TDF, EFV	>120	47	>100,000
S28	47	M	B	276	3TC, AZT, DRV/r, RAL	>120	24	584,000
S29	59	M	B	218	ETR, DRV/r, RAL	>200	84	—
S30	66	M	B	244	FTC, TDF, EFV	>189	182	—
S31	59	M	B	96	ABC, 3TC, DRV/r	91	51	—

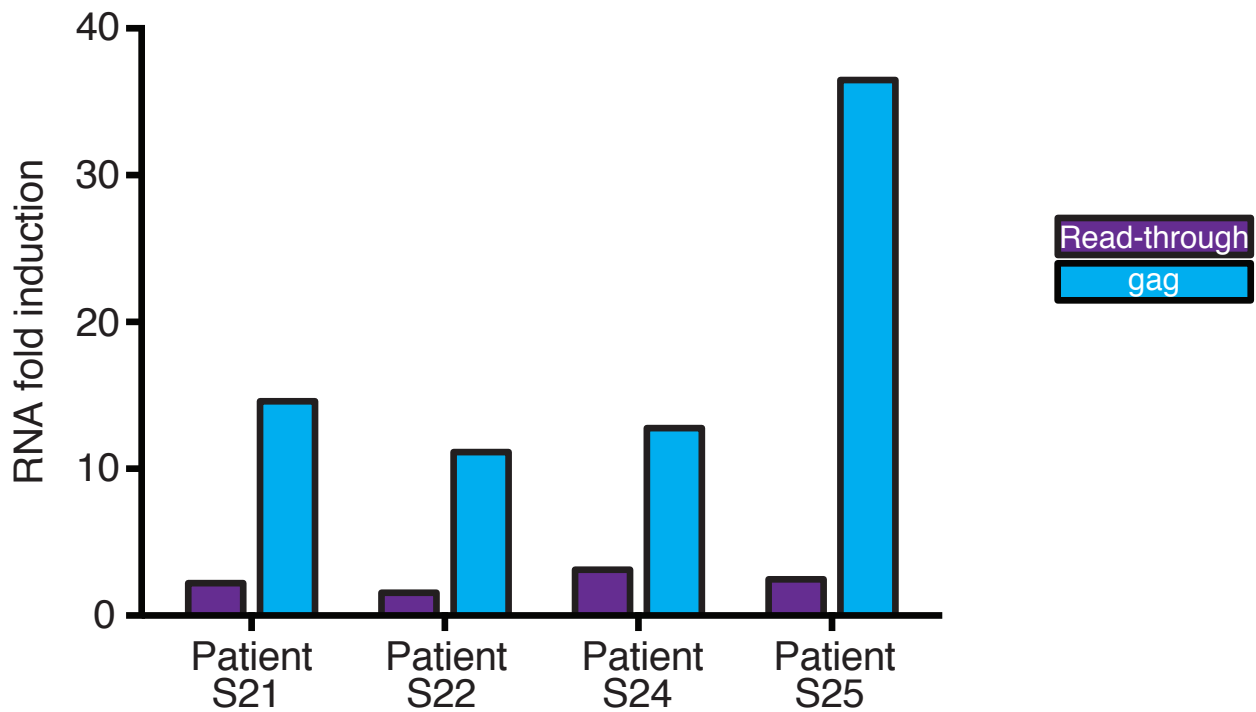
Abbreviations: abacavir (ABC), emtricitabine (FTC), lamivudine (3TC), tenofovir (TDF), efavirenz (EFV), etravirine (ETR), nevirapine (NVP), atazanavir boosted with ritonavir (ATV/r), darunavir boosted with ritonavir (DRV/r), fosamprenavir boosted with ritonavir (FPV/r), lopinavir boosted with ritonavir (LPV/r), elvitegravir boosted with cobicistat (EVG/c), raltegravir (RAL), maraviroc (MVC)



Supplementary Figure 1. MOLT-4/CCR5 cells do not induce allogeneic activation of rCD4s. rCD4s were isolated from an uninfected individual and stained with CFSE. Cells were then cultured alone, co-cultured with MOLT-4/CCR5 cells, or stimulated with anti-CD3 and anti-CD28 for 7 days. Cells were analyzed by flow cytometry for (a) CD25 expression, (b) morphology defined by forward scatter and side scatter, and (c) CFSE dilution indicative of activation-induced cell division.



Supplementary Figure 2. Short treatment with LRAs does not consistently induce HIV-1 mRNA production in cells from HIV-1 infected individuals on ART. Five million rCD4s from infected individuals on ART were cultured in triplicate with LRAs for 6 h. Intracellular HIV-1 mRNA from each replicate was measured using RT-qPCR. Mean effect of the LRA in each patient, depicted by individual dots, is presented as (a) fold change relative to DMSO control (mean \pm s.e.m.) and (b) copies of HIV-1 mRNA per million rCD4 equivalents. Statistical significance was determined using a paired t-test. Lines connect data points from individual subjects.



Supplementary Figure 3. PMA/I induces *bona fide* HIV-1 *gag* transcripts. Ten million rCD4s from five infected individuals on ART were cultured with DMSO alone or PMA/I for 6 h. DNase-treated intracellular RNA was measured by RT-qPCR. RT(-) controls were negative for all samples. Fold change relative to DMSO control is presented.