Supplemental Information

mTOR Overcomes Multiple Metabolic

Restrictions to Enable HIV-1 Reverse

Transcription and Intracellular Transport

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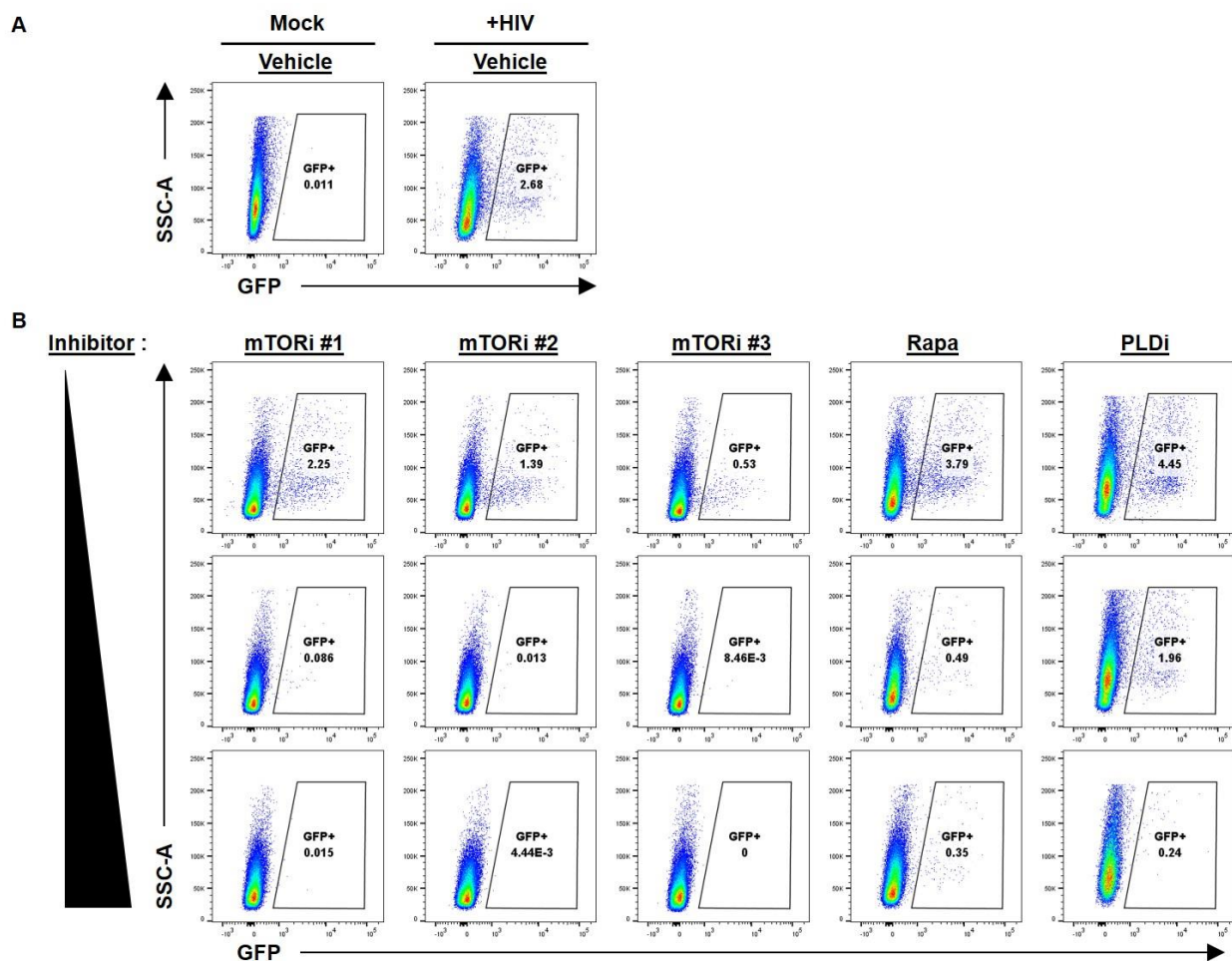
Figure S1. mTOR activity is essential for HIV-1 infection in TCR stimulated CD4 T cells. Related to Figure 1.

Representative flow cytometry data derived from Fig. 1A. (**A**) Resting primary CD4 T cells were stimulated for 48 h with anti-CD3/CD28 beads before infection with a X4-envelope-pseudotyped NL4-3-derived GFP reporter virus. Cells were cultured for three days post-infection and then analyzed for GFP expression by FACS. Gated infected (GFP+) cells are indicated. (**B**) Resting CD4 T cells were pretreated with increasing concentrations of catalytic mTOR inhibitors (mTORi #1, mTORi #2, mTORi #3), rapamycin, or PLDi for 24 h then stimulated as in (A) for 48 h with anti-CD3/CD28 beads before infection with a X4-envelope-pseudotyped NL4-3-derived GFP reporter virus. Cells were cultured in the continued presence of inhibitors for 3 days post-infection, and then analyzed for GFP expression by FACS.
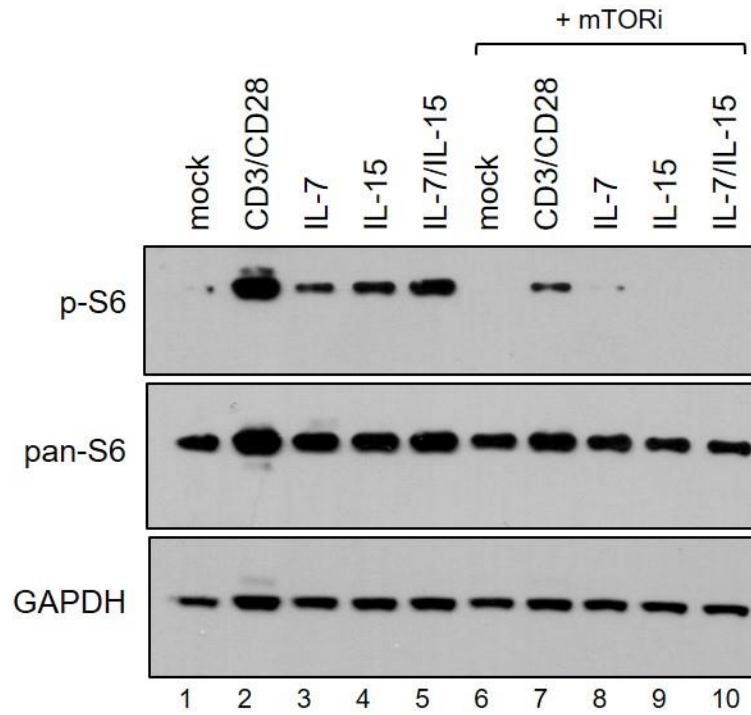
Figure. S2. Induction of mTOR activity in response to different stimuli. Related to Figure 1.

Whole-cell lysates (WCL) from CD4 T cells pretreated with mTORi as in Figure 1 and then stimulated with either anti-CD3/CD28 beads for 3 days or cytokines (IL-7, IL-15, or both) for 5 days were analyzed by immunoblotting. Data are representative of three independent donors.
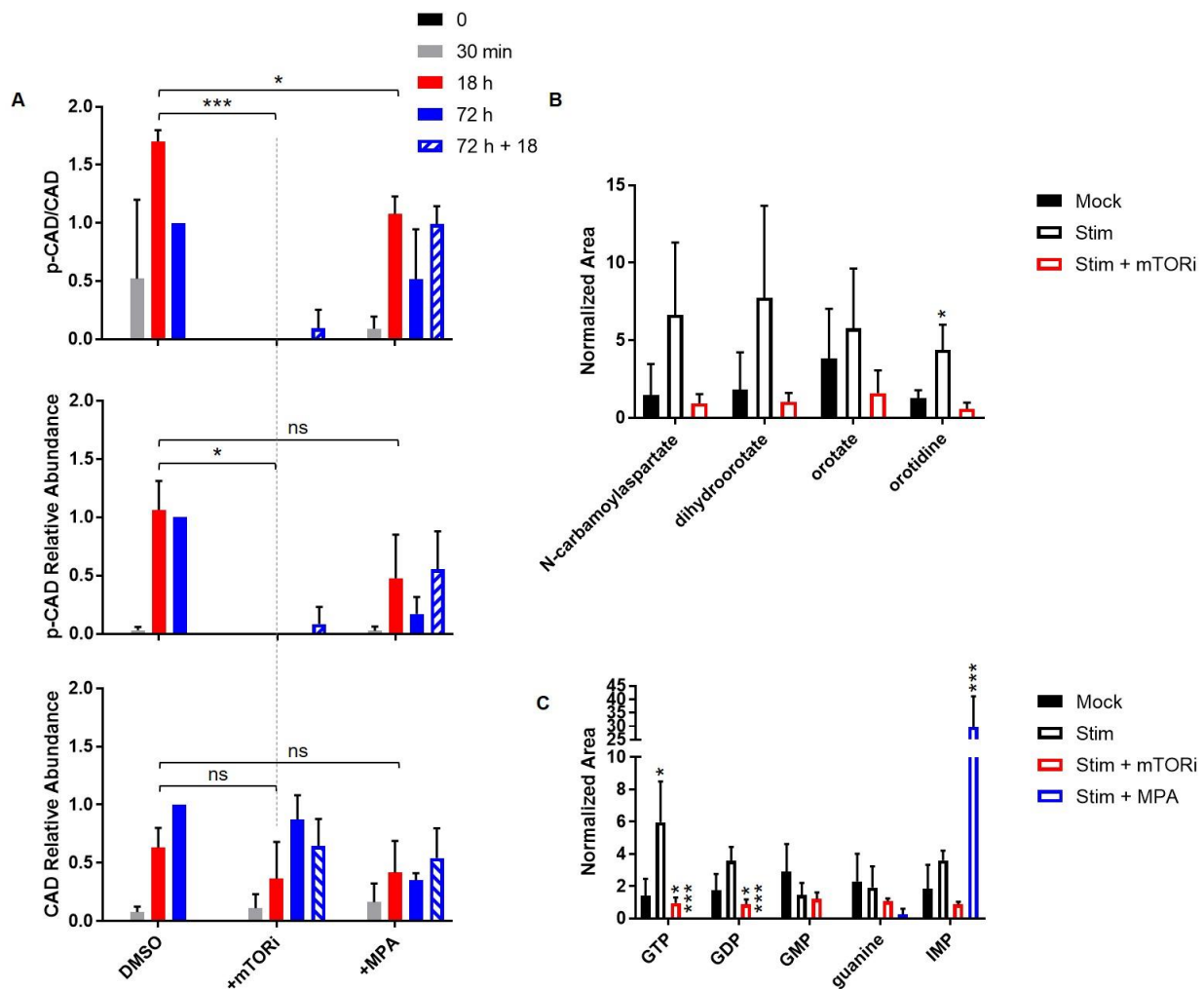
Figure S3. Pyrimidine and purine ribonucleotide pools are mTOR-dependent. Related to Figure 3.

(**A**) Data shown relate to Fig. 2C. Densitometry analysis of (p-CAD/GAPDH)/(CAD/GAPDH) ratio (top), p-CAD/GAPDH ratio (middle), and CAD/GAPDH ratio (bottom). Means and SDs of data are shown. Statistical significance of differences was determined by two-tailed Student's t tests *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$. n = 3. Global metabolomic analysis quantified pyrimidine (**B**) and guanine-containing purine (**C**), ribonucleotide metabolites in CD4 T cells stimulated with anti-CD3/CD28 beads for 48 h in the absence or presence of inhibitor pretreatment. Data were derived from CD4 T cells from three independent donors. Significant differences (Mock vs. Stim and Stim vs. Stim + mTORi or Stim + MPA) were determined by one-way analysis of variance (ANOVA). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$.
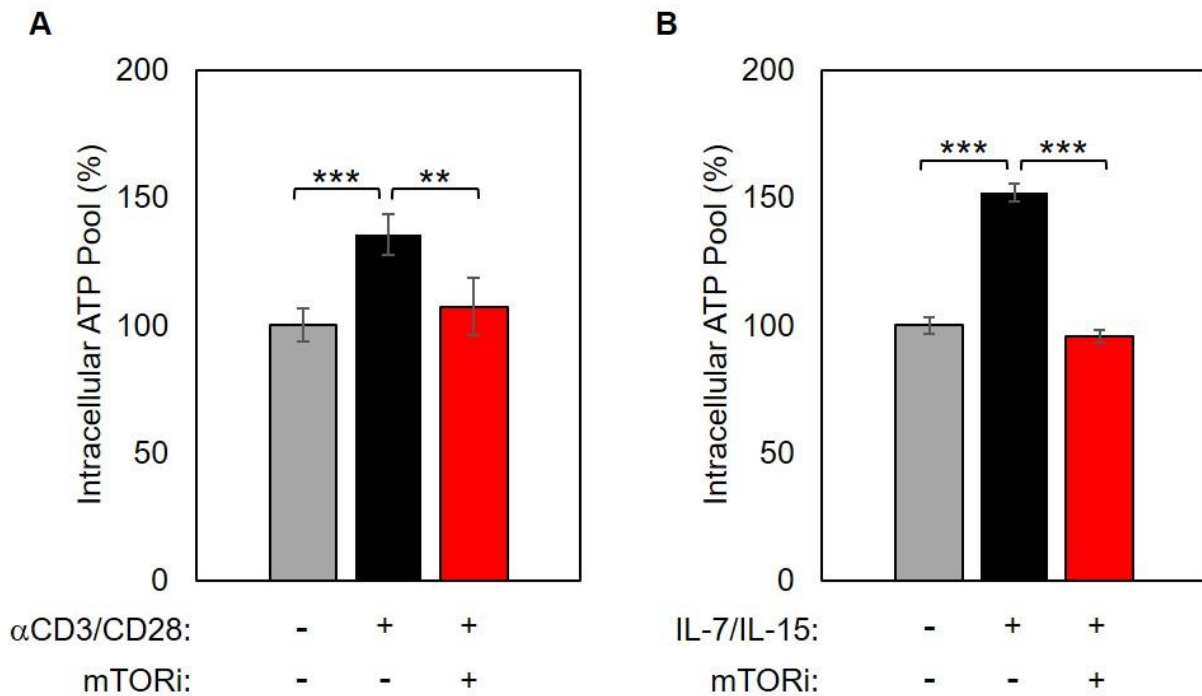
A

B

Figure S4. mTOR activity is essential for ATP pool expansion in γc cytokine stimulated CD4 T cells. Related to Figure 3.

CD4 T cells were stimulated with anti-CD3/CD28 beads for 24 h (**A**) or IL-7 plus IL-15 (100 ng/ml each) for 5 days (**B**) in the presence or absence of mTORi pretreatment. ATP concentrations were measured in cellular extracts. Data are representative of experiments from three independent donors. Means and SDs of data are shown. Statistical significance was determined by two-tailed Student's t tests. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$.
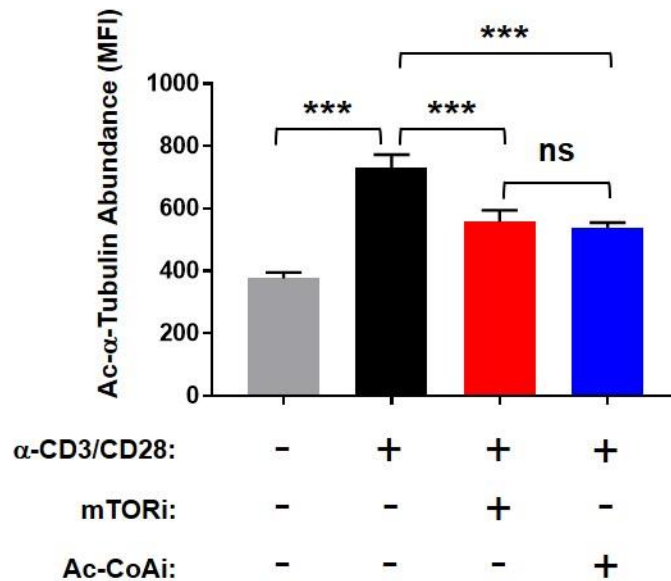
Figure S5. mTOR activity is essential for induction of acetylated Lys (40) α-tubulin (Ac-α-tubulin) after anti-CD3/CD28 stimulation. Related to Figure 3.

Sorted resting CD4 T cells from PBMCs were left untreated (grey bar) or stimulated with anti-CD3/CD28 beads for 48 hours in the absence (black bar) or presence of pretreatment with mTORi (red bar) or Ac-CoA synthesis inhibitors (ACLY and ACSS2 inhibitors) (blue bar). Cells were treated with inhibitors 24 h prior to stimulation and inhibition continued for duration of stimulation. The effects of inhibitors on accumulation of Ac-α-tubulin was determined by flow cytometry, using mean fluorescence intensity (MFI). Means and SDs of triplicate samples are shown. Statistical significance was determined by two-tailed Student's t tests. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.005$.

Table S1. Characteristics of Study Participants. Related to STAR Methods.

Subject ID	Sex	Age	Race & Ethnicity	Viral Load Status / (copies/ml)	BMI
1	M	22	Black	Viremic / >200K	20.8
2	M	25	Black	Viremic / >100K	39.2
3	M	25	Black	Viremic / >100K	22.0
4	M	28	Latino	Viremic / >2M	22.1
5	M	18	White	Viremic / >2M	27.6
6	M	21	Black	Viremic / >100K	27.8
7	M	24	Black	Viremic / >200K	27.3
8	M	26	Black	Viremic / >100K	22.2
9	M	19	Black	Viremic / >100K	19.6
10	M	21	Black	Viremic / >200K	23.4
11	M	21	Latino	Viremic / >200K	24.4
12	M	24	Latino	Viremic / >100K	20.5
13	M	22	Black	Viremic / >200K	23.3
14	M	25	Latino	ART-suppressed	22.5
15	M	25	Black	ART-suppressed	28.4
16	M	24	Black	ART-suppressed	19.6
17	M	25	Black	ART-suppressed	20.3
18	M	21	Latino	ART-suppressed	31.8
19	M	21	Black	ART-suppressed	23.5
20	M	21	Black	ART-suppressed	22.6
21	M	20	Black	ART-suppressed	23.6
22	M	25	Black	ART-suppressed	19.8
23	M	23	Latino	ART-suppressed	19.6
24	M	25	Black	ART-suppressed	23.9
25	M	25	Black	ART-suppressed	25.6
26	M	21	Latino	ART-suppressed	27.8