Supporting Information

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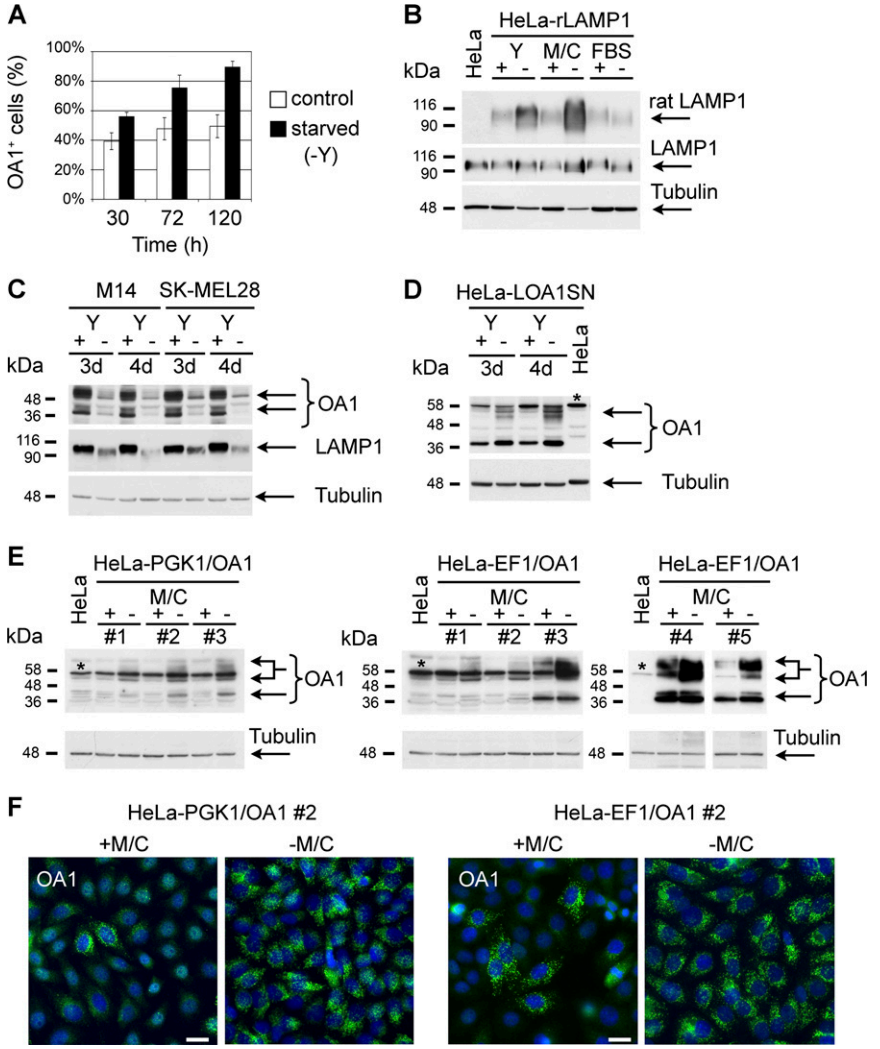

Fig. S1. Expression of different transgenes, driven by either viral or human promoters, is up-regulated by amino acid starvation. (*A*) Quantification of OA1-expressing cells in the HeLa-OA1myc population by immunofluorescence (IF) analysis, at the indicated times of tyrosine (Y) starvation. The results are expressed as a percentage of $OA1^+$ cells of the total cells (mean ± SD of 5–10 random fields from 1 experiment representative of 2). (*B*) Immunoblotting of lysates from HeLa cells stably transfected with an expression vector for rat LAMP1 and clonally selected (HeLa-rLAMP1) after 5 d of culture in the presence (+) or absence (–) of Y, methionine and cysteine (M/C), or FBS in the medium and decorated with specified Abs. Note the huge accumulation of exogenous rat LAMP1 protein on starvation without amino acids but not without FBS, compared with endogenous LAMP1 and tubulin. (*C*) Immunoblotting of lysates from M14 and SK-MEL28 melanoma cells after 3 and 4 d of Y starvation and decorated with specified Abs. Note that compared with the loading control (tubulin), the protein levels of both endogenous OA1 and LAMP1 do not increase but rather decrease on starvation. (*D*) Immunoblotting of lysates from HeLa cells stably transduced with a viral vector for OA1 (HeLa-LOA1SN) after 3 and 4 d of Y starvation and decorated with specified Abs. (*E*) Immunoblotting of different HeLa clones (#1–#5) stably expressing OA1 under either the human phosphoglycerate kinase 1 (PGK1) promoter (HeLa-PGK1/OA1) or the human elongation factor-1α (EF-1α) promoter (HeLa-EF1/OA1) after 3 d of culture in the presence (+) or absence (–) of M/C in the medium and decorated with specified Abs. Note the increase of exogenous OA1 protein on starvation. In *B*, *D*, and *E*, the cell lysate from nontransfected HeLa cells, not expressing rat LAMP1 or OA1, serves as a negative control for the specificity of the used Abs. *Nonspecific product recognized by the anti-OA1 Ab. (*F*) IF analysis of HeLa-PGK1/OA1 clone #2 and HeLa-EF1/OA1 clone #2 after 3 d of M/C starvation and stained with anti-OA1 Ab (green) and Hoechst (blue). The number of $OA1^+$ cells increases on starvation. (Scale bars: 20 μm.)

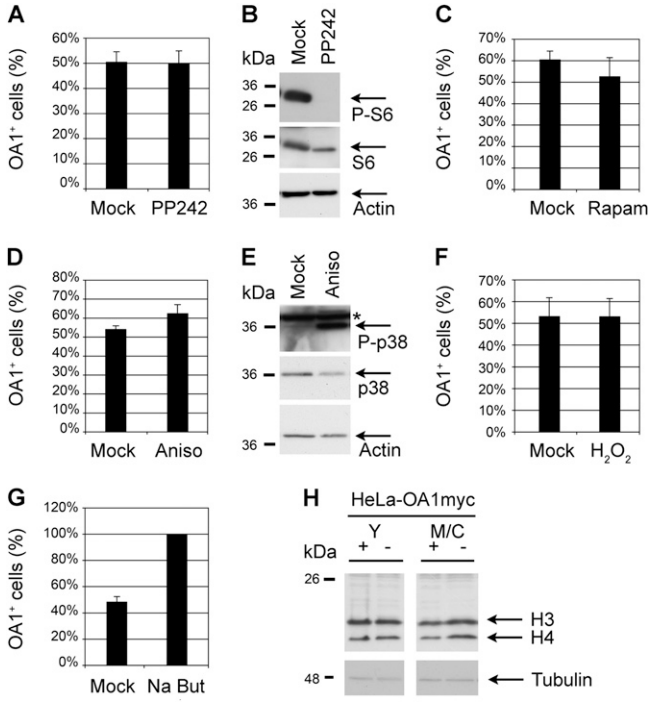

Fig. S2. Transgene up-regulation is not reproduced by mammalian target of rapamycin (mTOR) inhibition or by p38 activation but by histone hyperacetylation. HeLa-OA1myc cells were treated with PP242 (*A* and *B*) or rapamycin (Rapam; *C*) (mTOR inhibitors) for 2 and 5 d, respectively; with anisomycin (Aniso; *D* and *E*) or hydrogen peroxide (H_2O_2; *F*) (p38 activators) for 30 h; or with sodium butyrate (Na But; *G*) (pan-histone deacetylase inhibitor) for 24 h. (*A*, *C*, *D*, *F*, and *G*) Quantification of OA1-expressing cells in the HeLa-OA1myc population after the indicated treatments. The results are expressed as a percentage of $OA1^+$ cells of the total cells and represent the mean ± SEM of three independent experiments (*A* and *D*) or the mean ± SD of five random fields (*C*, *F*, and *G*). (*B* and *E*) Immunoblotting of HeLa-OA1myc cell lysates decorated with specified Abs to confirm treatment efficacy. *Nonspecific product recognized by the anti–phospho-p38 Ab. (*H*) Total histones H3 and H4 evaluated by immunoblotting of HeLa-OA1myc cell lysates after 3 d of culture in the presence (+) or absence (–) of tyrosine (Y) or methionine and cysteine (M/C) in the medium and decorated with specified Abs.

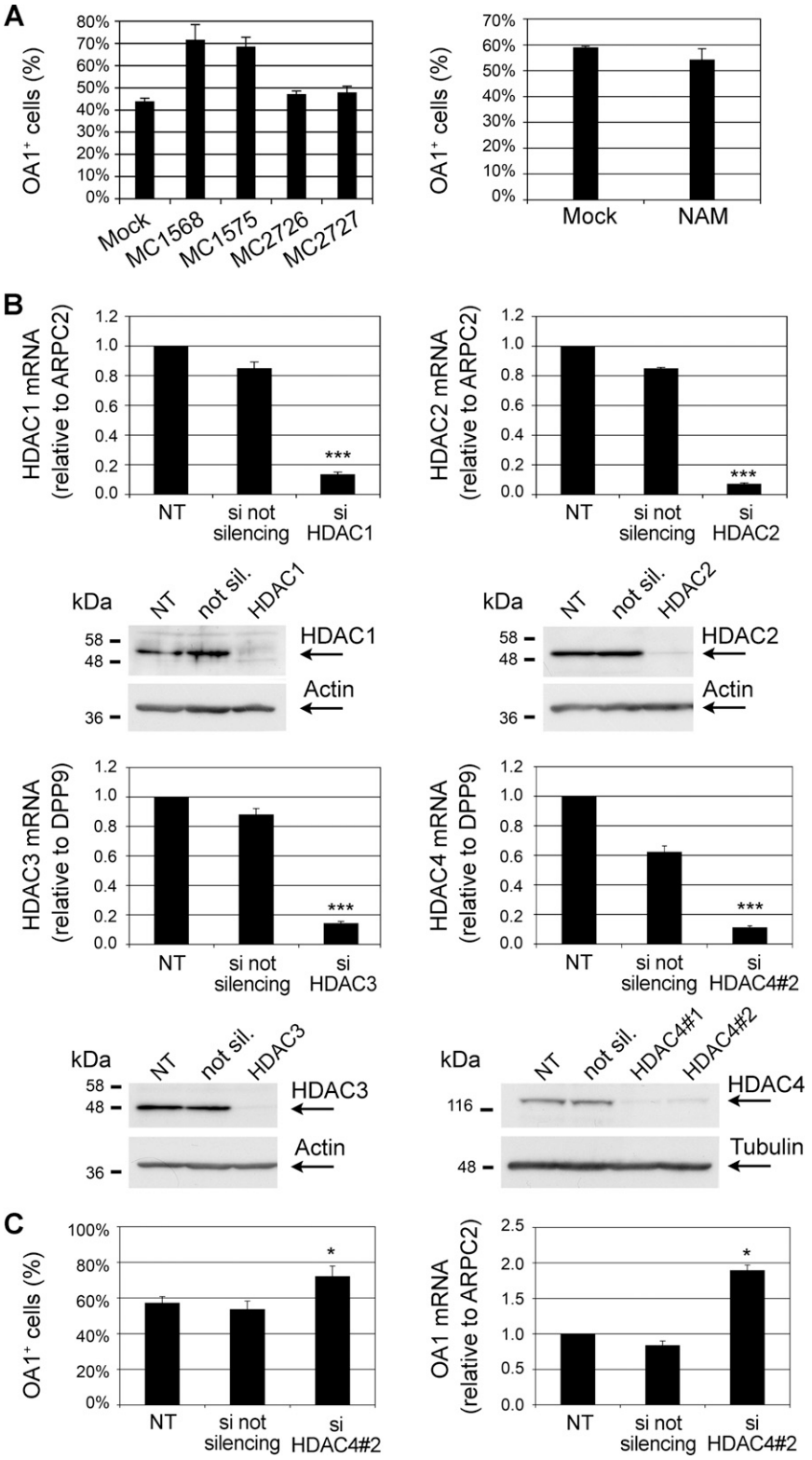

Fig. S3. Inhibition or knockdown of histone deacetylase 4 (HDAC4) but not of other HDACs or sirtuins leads to OA1 up-regulation. (*A*) Quantification of OA1-expressing cells in the HeLa-OA1myc population by immunofluorescence (IF) analysis after 5 d of the indicated treatments. MC1568 and MC1575 are class II HDAC inhibitors (HDACi); MC2726 and MC2727 are selective inhibitors of HDAC6 and of HDAC3/6, respectively; and nicotinamide (NAM) is a selective inhibitor of sirtuins. Results are expressed as a percentage of $OA1^+$ cells of the total cells [mean ± SD of 5 random fields from 1 experiment representative of 2 (*Left*) or mean ± SEM of 3 independent experiments (*Right*)]. (*B*) Quantification of HDAC1, HDAC2, HDAC3, and HDAC4 mRNA levels by real-time PCR following transfection with the corresponding siRNA (si) compared with the not silencing siRNA (si not silencing) or nontransfected (NT) cells. Data are expressed as the fold change vs. non-transfected cells (mean ± SEM of 5 independent experiments; $***P < 0.001$, paired two-tailed Student *t* test vs. not silencing siRNA). (*Lower*) Immunoblotting analysis of HDAC protein levels on whole-cell lysates after transfection with the corresponding siRNAs. Antitubulin or antiactin Abs were used as loading controls.

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(*C*) Assessment of OA1 expression in HeLa-OA1myc cells analyzed 72 h after transfection with siRNAs against HDAC4 (#2) or not silencing. (*Left*) IF quantification of OA1-expressing cells presented as a percentage of the total cells (mean ± SEM of 4 independent experiments; **P* < 0.05, paired two-tailed Student *t* test vs. nontransfected cells). (*Right*) Real-time PCR quantification of OA1 mRNA levels expressed as the fold change compared with the amount of the OA1 mRNA in nontransfected cells (mean ± SEM of 5 independent experiments; **P* < 0.05, paired two-tailed Student *t* test vs. nontransfected cells).

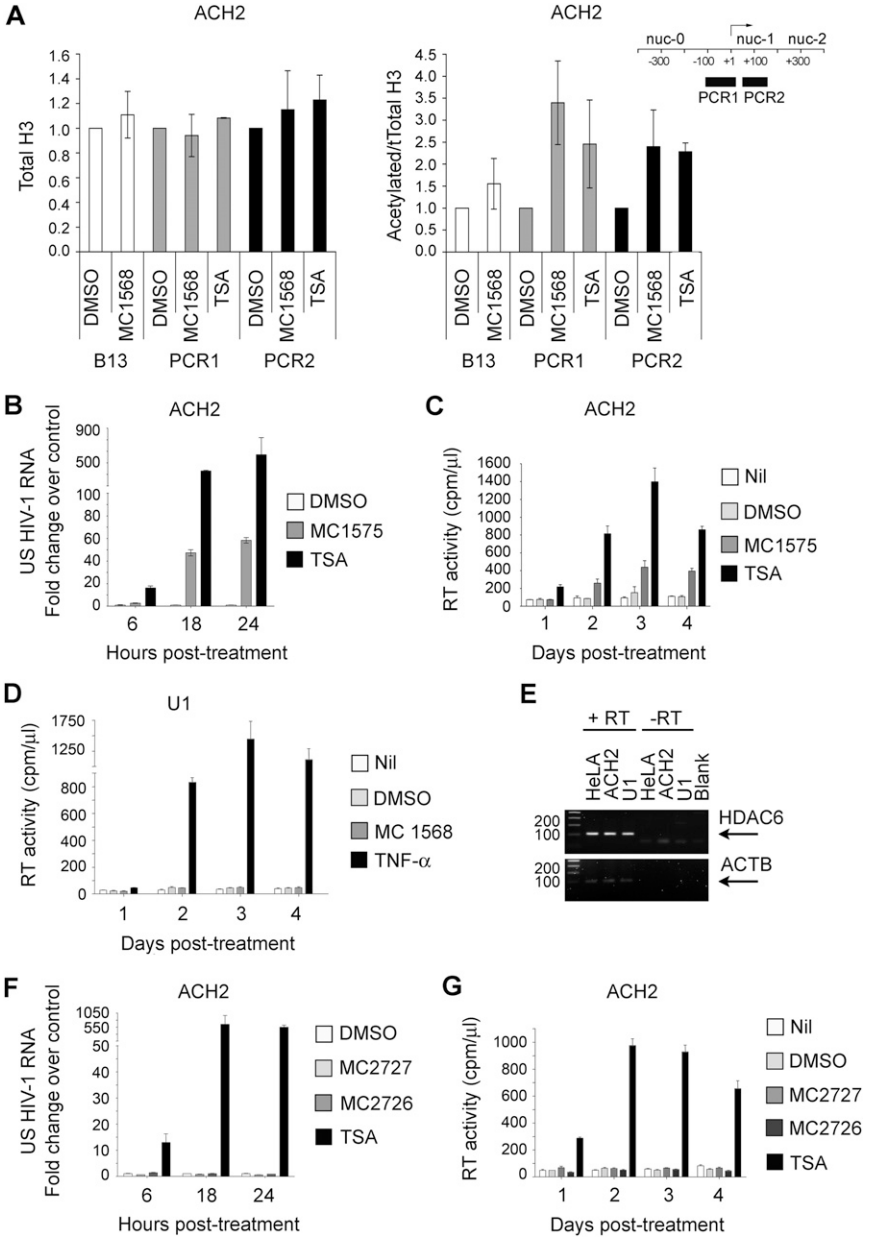

Fig. S4. Class II histone deacetylase (HDAC) inhibitors but not selective HDAC6 inhibitors reactivate latent HIV-1 in ACH-2 cells. (*A*) Quantification by ChIP and real-time PCR analysis of total and acetylated histone H3 bound to the LTR promoter after 24 and 18 h of MC1568 and trichostatin A (TSA) treatment, respectively, relative to control conditions. The scheme indicates the regions of the LTR promoter amplified by PCR1 and PCR2 primers (black bars), whereas B13 primers were used as a negative control. (*Left*) Results are normalized to the input DNA and expressed as the fold change vs. control (mean ± SEM of 2–3 independent experiments). (*Right*) Data represent the ratio of acetylated H3 vs. total H3 and are expressed as the fold change vs. control (mean ± SEM of 2–3 independent experiments). Although the amount of H3 associated with the LTR promoter does not change, its acetylation level increases after treatment. (*B*) Real-time PCR quantification of unspliced (US) HIV-1 RNA in ACH-2 cells incubated with DMSO (mock of MC1575), MC1575, or TSA at the reported times. Results are expressed as the fold change vs. the amount of US HIV-1 RNA in DMSO conditions at each time point (mean ± SEM of 3 technical replicates of 1 experiment representative of 2). (*C*) Reverse transcriptase (RT) activity of daily collected supernatants from ACH-2 cells incubated with standard medium (Nil), DMSO, MC1575, or TSA for up to 4 d (mean ± SEM of 3 technical replicates of 1 experiment representative of 3). (*D*) RT activity of daily collected supernatants from U1 cells incubated with standard medium, DMSO, MC1568, or TNF-α for up to 4 d (mean ± SEM of 2 independent experiments). (*E*) PCR amplification of HDAC6 and β-actin (ACTB) transcripts in HeLa, ACH-2, and U1 cell lines; HDAC6 is detected in ACH-2 and U1 cells. Blank, amplification in the absence of template. (*F*) Real-time PCR quantification of US HIV-1 RNA in ACH-2 cells incubated with DMSO (mock of MC2727 and MC2726), MC2727 (HDAC3–HDAC6 inhibitor), MC2726 (HDAC6 inhibitor), or TSA at the reported times. Results are expressed as the fold change vs. the amount of US HIV-1 RNA in DMSO conditions at each time point (mean ± SEM of 3 technical replicates). (*G*) RT activity of daily collected supernatants from ACH-2 cells incubated with standard medium, DMSO, MC2727, MC2726, or TSA for up to 4 d (mean ± SEM of 3 technical replicates).

Table S1. List of the siRNA duplexes

siRNA name	siRNA sequence
HDAC1	5′-ggcuccuaaaguaacaucatt-3′
HDAC2	5′-ccaccaugcuuuaugugautt-3′
HDAC3	5′-aaucagaacucacgccagutt-3′
HDAC4 #1	5′-cgacaggccucguguaugatt-3′
HDAC4 #2	5′-cgagcacugugguuuacaatt-3′
Not silencing	5′-gcaagaaguagaccggauatt-3′

HDAC4 siRNA indicated with #1 is described in Fig. 3, and HDAC4 siRNA indicated with #2 is an alternative duplex giving similar results and described in Fig. S3. HDAC, histone deacetylase.

Table S2. List of the primers for real-time PCR and conventional PCR

Primer name	Primer sequence	Conc.	T.	Eff.	Length
Primers used for real-time PCR using SYBR Green method (Roche Diagnostics) or conventional PCR					
OA1 F	5′-ctgatgccccatgaaaacc-3′	0.25	54	1.779	105
OA1 R	5′-tgtgctggcatcagaacc-3′				
ARPC2 F	5′-gtctgacttcctcaaggtgc-3′	0.3	54–56	1.814–2.010	153
ARPC2 R	5′-tccagtagcaagtgttccagc-3′				
DPP9 F	5′-gcaccacctgtggacagcc-3′	0.5	56	2.027	84
DPP9 R	5′-actgaccagcagagcgtgg-3′				
HDAC1 F	5′-ggaaatctatcgccctcaca-3′	0.5	56	1.896	168
HDAC1 R	5′-aacaggccatcgaatactgg-3′				
HDAC2 F	5′-taaatccaaggacaacagtgg-3′	0.5	56	1.956	89
HDAC2 R	5′-ggtgagactgtcaaattcagg-3′				
HDAC3 F	5′-tagacaaggactgagattgcc-3′	0.5	56	2.085	120
HDAC3 R	5′-gtgttagggagccagagcc-3′				
HDAC4 F	5′-ggtttattctgattgagaactgg-3′	0.5	56	1.977	146
HDAC4 R	5′-attgtaaaccacagtgctcgc-3′				
CMV prom. F	5′-aggcgttttgcgctgcttcg-3′	0.5	56	1.882	134
CMV prom. R	5′-aagttatgtaacgcggaactcc-3′				
CMV prom. F2	5′-cgtattagtcatcgctattacc-3′	0.5	56	1.853	97
CMV prom. R2	5′-gtggagacttggaaatcccc-3′				
ACTB prom. F	5′-gaggggggtaaaaaaatgctgc-3′	0.5	56	1.909	103
ACTB prom. R	5′-cgctcgagccataaaaggc-3′				
Primers used for real-time PCR using Taq man method (Applied Biosystems)					
gag sense	5′-acatcaagcagccatgcaaat-3′	0.9	60	1.938	104
gag antisense	5′-atctggcctggtgcaatagg-3′				
gag probe	5′-FAM-catcaatgaggaagctgcag gaatgggataga-TAMRA3′	0.2			
Primers used for conventional PCR					
ACTB F	5′-ccaaccgcgagaagatga-3′	1	56	/	97
ACTB R	5′-ccagaggcgtacagggatag-3′				
HDAC6 F	5′-gaatatcctttgcaactcccc-3′	1	56	/	105
HDAC6 R	5′-cctcaagcaggcaatgggc-3′				
CIDE-A F	5′-aagaggtcgggaatagcgagagtc-3′	1	58	/	305
CIDE-A R	5′-ctgcatccctatccacacgtgaacc-3′				

ACTB, β-actin; ARPC2, actin-related protein 2/3 complex, subunit 2; CIDE-A, cell death-inducing DFF45-like effector A; Conc., primer concentration (μM); DPP9, dipeptidyl-peptidase 9; Eff., primer efficiency; F, forward; HDAC, histone deacetylase; Length, amplicon length (bp); prom., promoter; R, reverse; T., annealing temperature (°C); /, not calculated.