Supporting Information

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SI Materials and Methods

Materials. We used two different siRNAs to target the mRNA of human SAMHD1 (NM_015474): FlexiTube siRNA Hs_SAMHD1_7 (Qiagen Cat. No. SI04243673) and siSAMHD1–3 siRNA reported in ref. 1. The AllStars Negative Control siRNA (Qiagen) was used as a negative control. The following antibodies were used: antihuman SAMHD1 mouse monoclonal clone [1A1] (ab128107; AbCam), antihuman R2 goat polyclonal (sc-10844; Santa Cruz Biotechnologies), antihuman p53R2 goat poyclonal (sc-10840; Santa Cruz Biotechnologies), antihuman R1 mouse monoclonal clone AD203 (MAB3033; Millipore), and antihuman glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) mouse monoclonal (MAB374; Chemicon International).

Transfections with siRNAs. In proliferating cells we analyzed the effects of SAMHD1 silencing by seeding 0.2×10^6 skin or lung fibroblasts in 10-cm dishes in DMEM with 10% (vol/vol) FCS and transfecting them after 24 h with 5 or 1 nM siRNA, respectively. In preliminary experiments, the two siRNAs mentioned above gave similar results both in terms of SAMHD1 down-regulation and inhibition of cell proliferation, as exemplified by results in Fig. 4. We used siRNA Hs_SAMHD1_7 for most other experiments. To obtain quiescent skin and lung fibroblasts, we seeded 0.4×10^6 cells per 10-cm dish in DMEM plus 10% (vol/vol) FCS and cultured them for 7 d until confluence. We then transfected the confluent cells with siRNAs (final concentration 5 nM for skin fibroblasts and 2 nM for lung fibroblasts) in DMEM with dialyzed 0.1% FCS without antibiotics using RNAiMAX (Life Technologies) according to the manufacturer's instructions. Three days later the medium was diluted 1:1 with fresh medium + 0.1% FCS, and the cells were kept for an additional 4 d in the presence of 2.5 nM or 1 nM siRNAs for skin and lung fibroblasts, respectively. The same cultures underwent a second transfection with 5 nM (skin fibroblasts) or 1 nM siRNAs (lung fibroblasts) in DMEM with 0.1% FCS, and the samples were analyzed 3 d later. By this protocol the cells remained in the presence of the siRNAs for a total of 10 d without replating.

RNA Extraction, Reverse Transcription, and Real-Time PCR. We quantified by real time RT-PCR the mRNA of SAMHD1 with the Applied Biosystems 7500 Real Time PCR System (Applied Biosystems).We extracted total RNA from 0.5×10^6 cells with TRIzol reagent (Invitrogen) and prepared cDNAs by reverse transcription. We performed real-time PCR assays in 96-well optical plates as described in ref. 2 using Quantitect Primer Assay kits (Qiagen) with specific primers for SAMHD1 and ribosomal protein large P0 (RPLP0) taken as endogenous control for normalizations.

Western Blotting. Pellets of 1–2 million cells were collected, washed with PBS, and lysed with radioimmunoprecipitation assay (RIPA) buffer (10 mM Tris·HCl pH 7.4, 100 mM NaCl, 1% sodium deoxycolate, 0.1% SDS, 1% Nonidet P-40) containing a mixture of protease inhibitors for mammalian cells (Sigma). The extract was centrifuged at 19,000 × *g* for 20 min, the protein concentrations of the supernatant solutions were determined by the BCA protein assay (Pierce), and appropriate amounts of the solution were loaded on precast gels (Bio-Rad) and electrophoresed. The proteins were blotted on Hybond-C extra (GE Healthcare) in the case of ribonucleotide reductase subunits R2 and p53R2, and GAPDH, and on PVDF for SAMHD1 (Millipore). Both membranes were saturated with 2% ECL Blocking Agent (GE Healthcare) for 1 h at room temperature and incubated overnight at 4 °C with the primary antibody (anti-SAMHD1 dilution 1:4,000, anti-R2 dilution 1:2,000, anti-p53R2 dilution 1:2,000, and anti-GAPDH 1:5,000). After three washings with PBS + 0.05% Tween 20 (T-PBS) for 10 min, the membranes were incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (dilution 1:80,000) for 1 h at room temperature. After further washing the membranes were developed using a chemiluminescence ECL kit (LiteAblotTurbo, Euroclone). The signals were detected on Kodak films and quantified with ImageJ software.

BrdU Incorporation. We grew 3.5×10^4 lung fibroblasts on glass coverslips in 3.5-cm dishes and transfected them after 24 h with negative control or anti-SAMHD1 siRNA. At different time points after transfection the cells were incubated with 30 μM BrdU for 30 min at 37 °C and fixed with 50 mM glycine, pH 2 + 70% ethanol for 20 min at –20 °C. We scored BrdU incorporation by immunofluorescence using the Roche Applied Science labeling and detection kit. Nuclei were counterstained with 0.2 μg/mL DAPI.

Immunofluorescence. Cells were grown in 35-mm-thin-bottomed Petri dishes for high-end microscopy (Ibidi), fixed with 4% paraformaldehyde for 15 min at 37 °C, permeabilized with 0.2% Triton X for 10 min at 37 °C, and blocked with MAXblock blocking medium (Active-Motif) for 1 h at 37 °C. The fixed cells were incubated with the indicated primary antibody for 1 h at 37 °C (anti-SAMHD1 dilution 1:200, anti-R2 dilution 1:200, anti-R1 dilution 1:100). After three 10-min washes with T-PBS, the cells were incubated with the appropriate secondary antibody (dilution 1:500) for 1 h at 37 °C. After washing with T-PBS the cells were counterstained with 0.2 μg/mL of DAPI in a mounting medium for fluorescence microscopy. The immunostained cells were visualized using a Leica TCS SP5 confocal microscope equipped with 63× oil immersion objective.

Analytical Procedures. Intracellular concentration of the four dNTPs were determined by an enzymatic assay (3) modified as described recently (4).

1. Laguette N, et al. (2011) SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. *Nature* 474(7353):654–657.
2. Rampazzo C, et al. (2007) Mitochondrial thymidine kinase and the enzymatic network regulating thymidine triphosphate pools in cultured human cells. *J Biol Chem* 282(48): 34758–34769.
3. Sherman PA, Fyfe JA (1989) Enzymatic assay for deoxyribonucleoside triphosphates using synthetic oligonucleotides as template primers. *Anal Biochem* 180(2):222–226.
4. Ferraro P, Franzolin E, Pontarin G, Reichard P, Bianchi V (2010) Quantitation of cellular deoxynucleoside triphosphates. *Nucleic Acids Res* 38(6):e85.

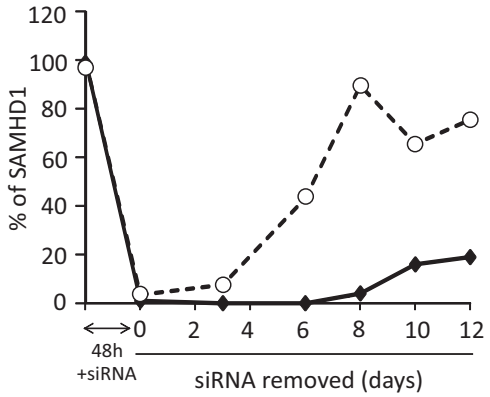
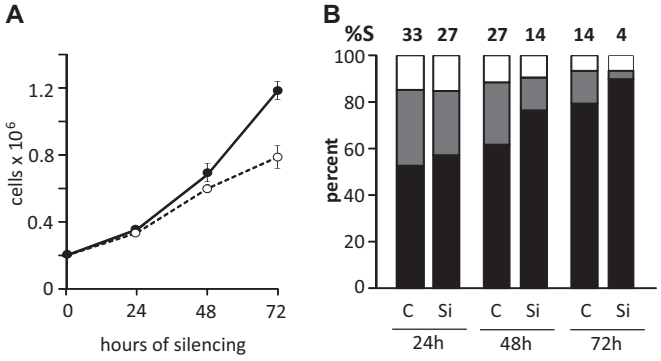


Fig. S1. Decline and recovery of SAMHD1 mRNA and protein in WT skin fibroblasts during siRNA transfection and subsequent removal of siRNA from the medium. Proliferating cultures of skin fibroblasts were transfected for 48 h with anti-SAMHD1 siRNA 24 h after seeding. Cells were then replated in siRNA-free medium and grown for 12 d with medium changes every 3–4 d. SAMHD1 mRNA (open circle) and protein (filled diamond) were measured at the indicated times.



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		dNTP pmol/10^6 cell			
		dGTP	dTTP	dATP	dCTP
48h	control	12,3 ± 1,4	57,4 ± 2,1	28,6 ± 5,5	29,7 ± 2,2
	silenced	30,3 ± 2,9	100,5 ± 10,9	52,2 ± 16,8	44,2 ± 3,9
72h	control	5,7 ± 0,2	29,3 ± 0,9	13,1 ± 0,5	15,6 ± 0,3
	silenced	31,1 ± 6,1	75,0 ± 8,1	42,9 ± 4,3	31,8 ± 3,3

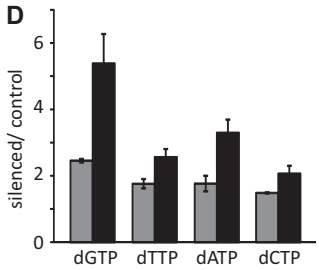

Fig. S2. Effect of SAMHD1 silencing on the growth of WT skin fibroblasts. (*A*) Cell growth in cultures transfected for 24–72 h with anti-SAMHD1 (open circle) or control (filled circle) siRNAs. Data are from three experiments. Bars indicate SEs. (*B*) Percent of G1-phase (black), S-phase (gray), and G2/M-phase (white) cells in transfected cultures. The frequency of S-phase cells is indicated above each control (C) and silenced (Si) sample. (*C*) Sizes of dNTP pools from WT skin fibroblasts transfected for 48 or 72 h with anti-SAMHD1 or control siRNAs. (*D*) Ratios of pool sizes in silenced and control cultures after 48 (gray) or 72 (black) h of transfection. Data are from three experiments. Bars indicate SEM.

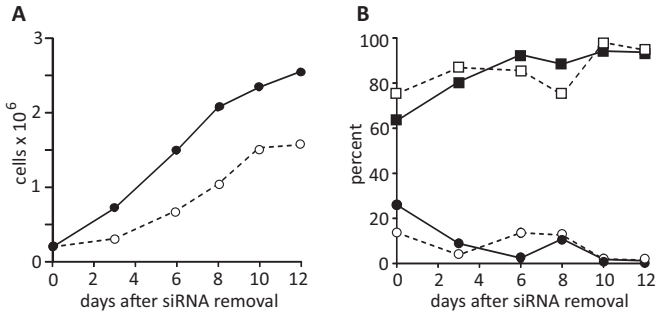

Fig. S3. Slow recovery of growth in WT skin fibroblasts after SAMHD1-silencing. (*A*) Cell growth of control (filled circle) and SAMHD1-silenced (open circle) skin fibroblasts replated in siRNA-free medium after 48 h transfections. (*B*) Frequencies of G1- (squares) and S-phase (circles) cells in control (filled square, filled circle) and silenced (open square, open circle) cultures. Data from one experiment carried out as in Fig. 6.

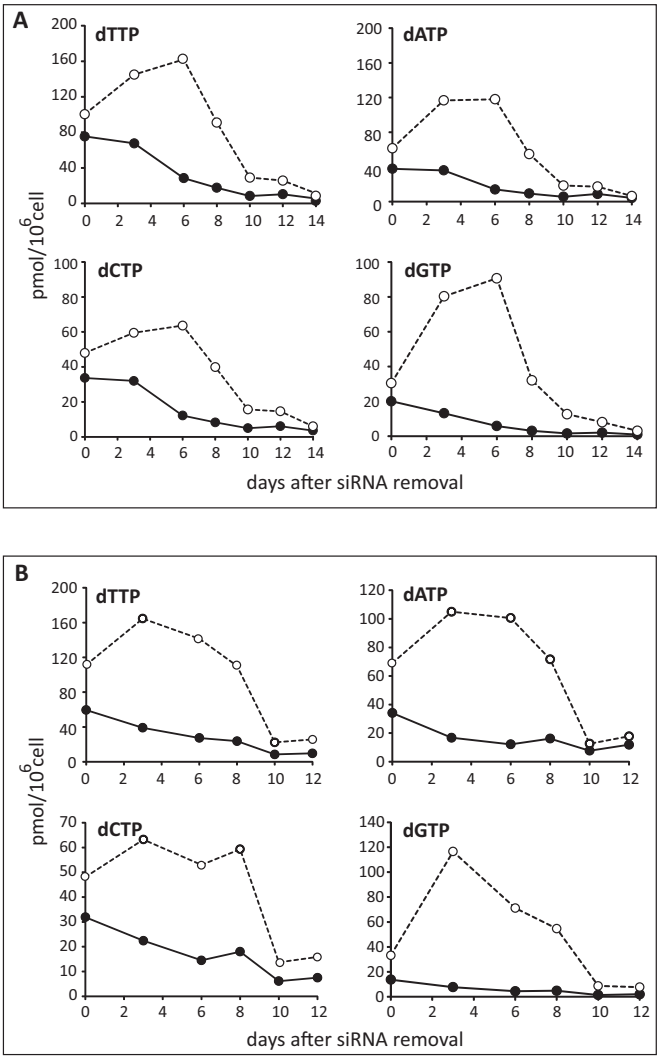

Fig. S4. Sizes of dNTP pools in lung and WT skin fibroblasts after siRNA-silencing of SAMHD1. (*A*) Pool sizes in lung fibroblasts during the recovery period in the absence of siRNAs. Data are from the experiment in Fig. 6*C*. (*B*) Pool sizes in WT skin fibroblasts during the recovery period in the absence of siRNAs. Data are from the experiment in Fig. S3. Filled circle, control; open circle, SAMHD1-silenced samples.

Table S1. Sizes of the four dNTP pools in human lung and skin fibroblasts transfected with control or anti-SAMHD1 siRNAs during 10 d of serum starvation

		dNTP pmol/10^6·cell*			
Cell line	Treatment	dGTP	dTTP	dATP	dCTP
Lung fibroblasts	Control	0.8 ± 0.1	4.2 ± 0.7	2.6 ± 0.4	3.5 ± 0.4
	Silenced	4.4 ± 0.5	10.7 ± 1.9	8.2 ± 0.7	8.2 ± 0.8
WT skin fibroblasts	Control	0.5 ± 0.1	2.4 ± 0.4	2.4 ± 0.5	2.9 ± 0.5
	Silenced	2.4 ± 0.5	5.4 ± 0.8	4.5 ± 1	5.0 ± 0.9
p53R2$^-$ skin fibroblasts	Control	0.4 ± 0.1	8.3 ± 2.2	1.3 ± 0.2	1.7 ± 0.1
	Silenced	0.8 ± 0.1	16.8 ± 4.2	2.3 ± 0.5	2.1 ± 0.4

*Values are means ± SEM of measurements from four to six experiments per cell line.