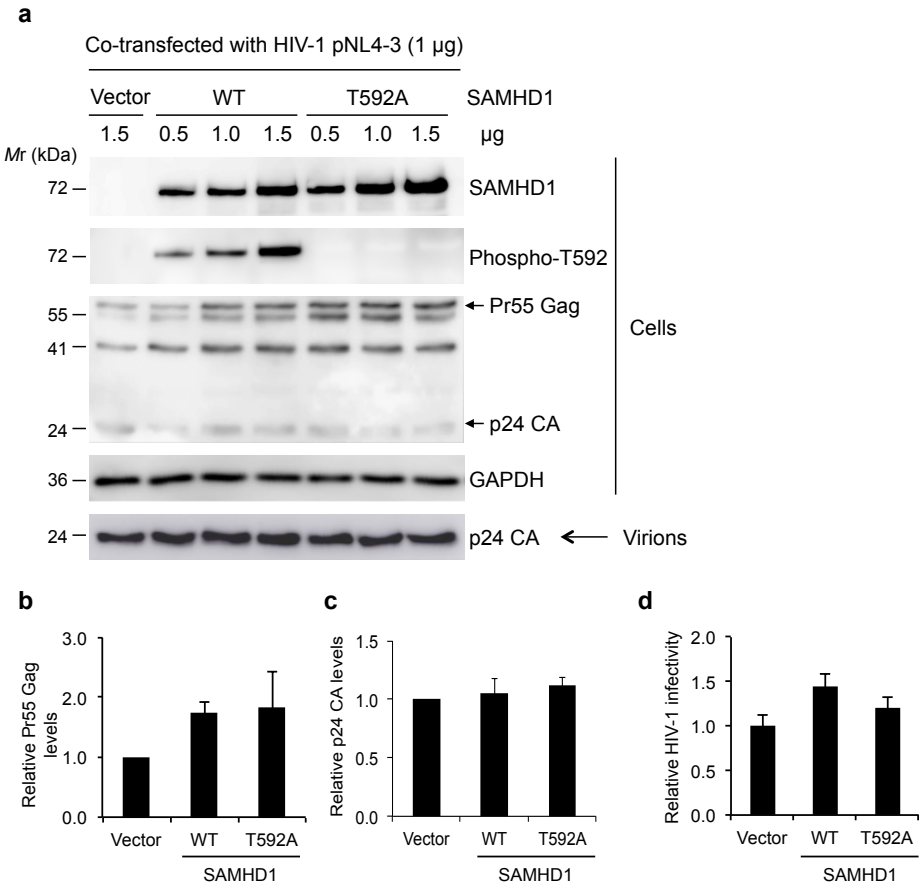
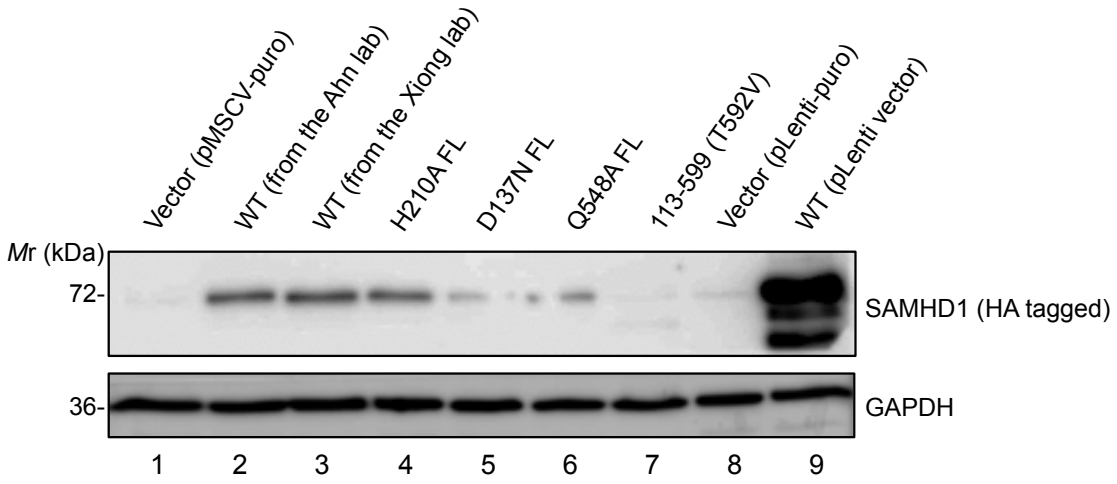
Supplementary Fig. 1. Overexpression of SAMHD1 in HEK293T cells does not reduce HIV-1 Gag protein synthesis and viral particle release.



(**a**) HEK293T cells were co-transfected with the HIV-1 proviral DNA pNL4-3 and increasing amounts of SAMHD1 wild-type (WT) or a phospho-ablative mutant SAMHD1^{T592A} expressing plasmid. Cell supernatants containing released virions were clarified and pelleted via ultracentrifugation. The pelleted virions were lysed for analysis by immunoblotting to determine the level of p24 capsid (CA). The transfected HEK293T cells were treated with subtilisin to remove plasma membrane-bound viral particles. Cells were lysed and the expression levels of SAMHD1, HIV-1 gag, and p24 CA were determined by immunoblotting. Thr592-phosphorylated SAMHD1 (phospho-T592) was determined using specific antibodies. GAPDH was used as a loading control. Blot is

representative of three independent experiments. (**b and c**) Semi-quantitative analysis of the relative levels of Pr55 gag protein in cells (**b**) and p24 CA in virions (**c**) in the absence or presence of SAMHD1 WT and SAMHD1^{T592A} protein expression. Image J software was used to calculate the densitometry of cellular Pr55 gag, virion p24 CA and GAPDH bands in three independent experiments including that represented in (**a**). For normalization, the densitometry of the intracellular Pr55 Gag band in each lane was normalized to the densitometry of the corresponding GAPDH band. The normalized densitometry of the Pr55 gag band and the p24 CA band in the vector control sample was set as 1 in (**b**) and (**c**), respectively. Error bars represent the standard deviation of triplicate experiments. (**d**) The relative infectivity of HIV-1 virions generated from transfected HEK293T cells in the presence of SAMHD1 was determined using a reporter cell line and normalized to the vector control cells (set as 1). There is no statistical difference compared among vector control, WT SAMHD1, and the SAMHD1^{T592A} mutant ($p \geq 0.08$) in (**b–d**). Statistical analysis was performed using a Student's *t*-test.

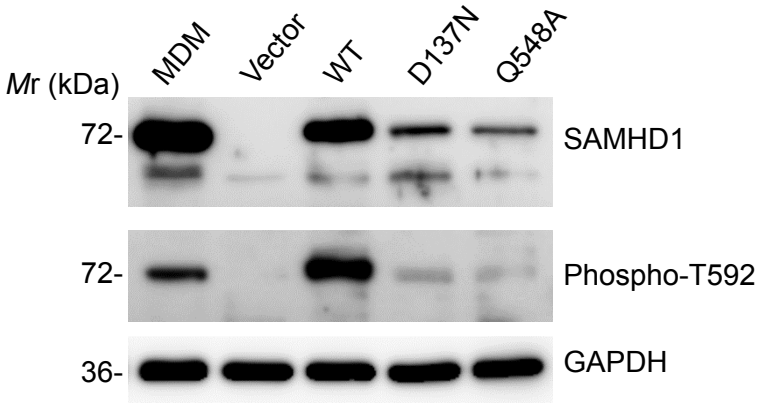
Supplementary Fig. 2. Expression of wild-type and mutant SAMHD1 proteins by different constructs in PMA-differentiated U937 cells.



- Lanes #1-7: pMSCV-puro vector and its derived SAMHD1 constructs;
- Lanes #1-2: pMSCV-puro vector and WT SAMHD1 construct from the Ahn lab.
- Lanes #3-7: SAMHD1 constructs generated by the Xiong lab
- Lanes # 8-9: pLenti-puro vector and it derived construct (from the Landau lab)
- FL: full-length

U937 cells were transduced by pLenti-puro of pMSCV-puro vectors expressing wild-type (WT) or mutant SAMHD1. Two separated clones of pMSCV expressing WT SAMHD1 were tested (from the labs of K. Ahn or Y. Xiong, respectively). The result shows a long exposure and there were multiple bands for pLenti-expressed WT SAMHD1. Cells were differentiated with PMA and lysed and the expression levels of SAMHD1 were determined by immunoblotting with anti-HA antibodies. GAPDH was used as a loading control. FL, full-length. The data shown are representative of three independent immunoblotting experiments.

Supplementary Fig. 3. The levels of SAMHD1 wild-type and mutants expressed in differentiated U937 cells are lower than that of primary monocyte-derived macrophages.



Monocyte-derived macrophages (MDM) were differentiated from monocytes of a healthy donor using M-CSF for 7 days, at which point protein lysates were harvested. Stable U937 cell lines expressing SAMHD1 wild-type (WT) or mutants were differentiated with PMA (30 ng/ml) for 20 h prior to harvesting protein lysates. Total protein was quantified and 10 µg of each lysate was analyzed by immunoblotting. Membranes were probed with a SAMHD1-specific antibody and a phospho-specific SAMHD1 antibody recognizing phosphorylation at Thr592. GAPDH was used as a loading control. The data shown are representative of three independent immunoblotting experiments.