SAMHD1 Promotes DNA End Resection to Facilitate DNA Repair by Homologous Recombination

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Graphical Abstract

Highlights
- SAMHD1 deficiency or Vpx-mediated degradation sensitizes cells to DSB-inducing agents
- SAMHD1 localizes to DNA double-strand breaks in response to DNA damage
- SAMHD1 promotes HR and DNA end resection independent of its dNTPase activity
- SAMHD1 complexes with CtIP and facilitates its recruitment to DNA damage sites

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In Brief
SAMHD1 is a dNTP triphosphohydrolase, which restricts HIV-1 infection and is dysregulated in Aicardi-Goutières syndrome and cancer. Daddacha et al. define a dNTPase-independent function for SAMHD1 in HR-mediated DSB repair by facilitating CtIP accrual to promote DNA end resection, providing insight into how SAMHD1 promotes genome integrity.

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SAMHD1 Promotes DNA End Resection to Facilitate DNA Repair by Homologous Recombination

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SUMMARY

DNA double-strand break (DSB) repair by homologous recombination (HR) is initiated by CtIP/MRN-mediated DNA end resection to maintain genome integrity. SAMHD1 is a dNTP triphosphohydrolase, which restricts HIV-1 infection, and mutations are associated with Aicardi-Goutières syndrome and cancer. We show that SAMHD1 has a dNTPase-independent function in promoting DNA end resection to facilitate DSB repair by HR. SAMHD1 deficiency or Vpx-mediated degradation causes hypersensitivity to DSB-inducing agents, and SAMHD1 is recruited to DSBs. SAMHD1 complexes with CtIP via a conserved C-terminal domain and recruits CtIP to DSBs to facilitate end resection and HR. Significantly, a cancer-associated mutant with impaired CtIP interaction, but not dNTPase-inactive SAMHD1, fails to rescue the end resection impairment of SAMHD1 depletion. Our findings define a dNTPase-independent function for SAMHD1 in HR-mediated DSB repair by facilitating CtIP accrual to promote DNA end resection, providing insight into how SAMHD1 promotes genome integrity.

INTRODUCTION

DNA double-strand breaks (DSBs) are cytotoxic lesions induced by exogenous sources, such as ionizing radiation (IR), and endogenous sources, such as replication stress and meiotic recombination. Failure to repair DSBs leads to cell death or mutagenic events that drive genomic instability. Indeed, DSB repair defects are associated with cancer, premature aging, neurodegeneration, infertility, and developmental and immunological abnormalities (Jackson and Bartek, 2009). DSBs are repaired predominantly by two distinct, but highly coordinated pathways: error-prone non-homologous end joining (NHEJ), which involves direct ligation of broken DNA ends and error-free homologous recombination (HR), which involves an intact copy of the damaged site (Symington and Gautier, 2011). Whereas NHEJ operates throughout the cell cycle, HR functions primarily in S/G2 phase, when a sister chromatid is available as a repair template. HR is initiated by DNA end resection in which processing of the 5' ends of DSBs by the CtBP-interacting protein (CtIP) endonuclease (Limbo et al., 2007; Makharashvili et al., 2014; Sartori et al., 2007; Wang et al., 2014; You et al., 2009), together with the MRE11-RAD50-NBS1 (MRN) endo and 3' to 5' exonuclease complex (Anand et al., 2016; Cannavo and Cejka, 2014; Garcia et al., 2011; Nicolette et al., 2010; Paul and Gellert, 1998; Stracker and Petriti, 2011), generates short 3' single-stranded DNA (ssDNA) overhangs, which are further extended by the EXO1 or DNA2 nucleases together with the BLM or WRN helicase (Cejka et al., 2010; Gravel et al., 2008; Mimitou and Symington, 2008; Nimonkar et al., 2011; Niu et al., 2010; Zhu et al., 2008). The 3' ssDNA overhangs are bound by RPA, which is then displaced by RAD51 to form a RAD51-ssDNA nucleoprotein filament with the assistance of mediator proteins, including BRCA2, to mediate HR (Prakash et al., 2015). RPA-ssDNA also recruits ATRIP to activate the ATR checkpoint kinase (Zhang et al., 2016; Zou and Elledge, 2003). Thus, DNA end resection is a critical determinant of DNA repair pathway choice and checkpoint activation.

Sterile alpha motif and histidine-aspartic acid (HD) domain-containing protein 1 (SAMHD1) is a deoxyribonucleoside triphosphate (dNTP) triphosphohydrolase (Goldstone et al., 2011; Powell et al., 2011) with a well-defined role in restricting HIV type 1 (HIV-1) and other viral infections, particularly in non-dividing cells by depleting dNTPs required for reverse transcription and replication (Baldauf et al., 2012; Hrecka et al., 2011; Lagouette et al., 2011; Laouassa et al., 2012). Mutations in SAMHD1 also cause Aicardi-Goutières syndrome (AGS) (Rice et al., 2009), a congenital neurodegenerative autoimmune disorder, and, moreover, SAMHD1 is recurrently mutated in chronic lymphocytic leukemia (CLL) (Clifford et al., 2014), frequently...
mutated in colorectal cancer (Rentoft et al., 2016), as well as mutated or downregulated in a number of other cancers (Kohnken et al., 2015), suggesting that SAMHD1 functions as a tumor suppressor. SAMHD1 contains a SAM domain, a protein interaction module (Schulte et al., 1997) and a HD domain, found in a superfamily of proteins with metal-dependent phosphohydrolase activity (Avrind and Koonin, 1998). In addition to its well-established dNTPase activity, SAMHD1 binds to ssDNA/RNA (Beloglazova et al., 2013; Goncalves et al., 2012; Seamon et al., 2015, 2016; Tüngler et al., 2013) at its dimer-dimer interface, which sterically blocks tetramerization (Seamon et al., 2016) required for its dNTPase activity (Brandariz-Nuñez et al., 2013; Seamon et al., 2013; Ji et al., 2014; Yan et al., 2013; Zhu et al., 2013), and SAMHD1 has been reported to possess Dnase/RNase activity (Beloglazova et al., 2013; Ryoo et al., 2014), however, a number of studies indicate that SAMHD1 lacks active-site-associated nuclease activity (Antonucci et al., 2016; Goldstone et al., 2011; Goncalves et al., 2012; Seamon et al., 2015, 2016; Welbourn and Strebel, 2016), which has been attributed to persistent co-purifying contaminants (Antonucci et al., 2016; Seamon et al., 2015).

SAMHD1 has been shown to promote genome integrity by maintaining dNTP pool balance through its dNTPase activity (Franzolin et al., 2013; Kohnken et al., 2015; Kretschmer et al., 2015; Clifford et al., 2014; Rentoft et al., 2016). Increased spontaneous DNA damage and dNTP pools was observed in cells from AGS patients with SAMHD1 dysregulation (Kretschmer et al., 2015), and SAMHD1 depletion in cells leads to dNTP pool imbalance in cycling cells (Franzolin et al., 2013). Moreover, several heterozygous colorectal cancer-associated mutations impair SAMHD1’s dNTPase activity, and elevated dNTP pools in combination with inactivated mismatch repair increase mutation rates, suggesting that heterozygous cancer-associated SAMHD1 mutations increase mutation rates in cancer cells (Rentoft et al., 2016). Consistent with these findings, SAMHD1 overexpression in cells causes DNA damage hypersensitivity, however, somewhat paradoxical to its role in dNTP pool regulation, overexpressed SAMHD1-HA localizes to DNA damage sites (Clifford et al., 2014). How SAMHD1 functions to promote genome integrity is unclear. Here, we show that SAMHD1 has an unexpected dNTPase-independent function in promoting DNA end resection to facilitate DSB repair by HR through Ctp recruitment to DNA damage sites.

**RESULTS**

**SAMHD1 Functions in DNA DSB Repair**

To determine the role of SAMHD1 in responding to DNA damage, we examined U2OS cells depleted for SAMHD1 for sensitivity to IR, etoposide, and camptothecin (CPT), which directly or indirectly induce DSBs. Two small interfering RNAs (siRNAs) targeting SAMHD1 caused IR, CPT, and etoposide hypersensitivity compared to a non-targeting (NT) control (Figures 1A–1C), implying that SAMHD1 responds to DSBs. Western blot analysis confirmed SAMHD1 knockdown in these cells (Figure 1D). A similar CPT hypersensitivity following SAMHD1 depletion was observed in MCF7 cells, which could be rescued by expression of exogenous SAMHD1-GFP (Figures 1E and 1F), non-tumorigenic BEAS-2B cells, (Figures S1A and S1B), and was also observed in HCT-116 SAMHD1 knockout (KO) cells (Figures S1C and S1D), suggesting that the phenotype is not cell-type specific, is not due to an off-target effect, and that SAMHD1-GFP is functional for alleviating DSB-inducing agent sensitivity. To provide direct evidence that SAMHD1 responds to DSBs at the single-cell level, we performed a neutral comet assay in U2OS cells depleted for SAMHD1 and treated with IR. SAMHD1 depletion in cells caused a significant delay in repair of IR-induced DSBs, as measured by comet tail moment compared to a NT control in cells synchronized in S phase (Figures 1G, 1H, and S1E), but not in unsynchronized cells (Figures S1E–S1G), suggesting that SAMHD1 promotes DSB repair predominantly in S phase, where HR is dominant.

**SAMHD1 Localizes to DSBs in Response to DNA Damage**

Overexpressed SAMHD1-HA has been reported to localize to DNA damage sites in response to CPT treatment (Clifford et al., 2014). To determine if endogenous SAMHD1 behaves similarly and localizes to DSBs, we analyzed SAMHD1 accumulation at DNA damage sites in response to IR and CPT treatment in HeLa cells. A significant increase in percent of cells with endogenous SAMHD1 foci was observed following IR and CPT treatment (Figure 2A), which co-localized with γH2AX, a marker for DSBs (Figure 2B), and RAD51, a marker for HR (Figure 2C), suggesting that SAMHD1 localizes directly to DSBs in response to DNA damage. Both endogenous SAMHD1 and SAMHD1-GFP expressed in U2OS cells also localized to DNA damage sites induced by laser microirradiation, which co-localized with RPA70, a marker for ssDNA formed by DSB end resection (Figures 2D and S2). To determine if endogenous SAMHD1 localizes to nascent DNA (naDNA) at CPT-induced one-sided DSBs and rule out co-localization resulting from random events, we used single-molecule super-resolution (SR) microscopy (Whelan et al., 2016) on U2OS cells pulse labeled with EdU and treated with or without CPT. Similar to CtIP, a significant increase in co-localization of SAMHD1 with naDNA above random levels was observed following CPT treatment (Figures 2E and 2F), suggesting that SAMHD1 localizes directly to replication-associated DSBs.

**SAMHD1 Functions in DSB Repair by Facilitating HR**

Poly ADP-ribose polymerase (PARP) inhibitor sensitivity is associated with defects in HR (Helleday et al., 2005). Indeed, SAMHD1 depletion in U2OS, MCF7, and primary small airway epithelial cells caused hypersensitivity to veliparib, a PARP inhibitor (Figures 3A, 3B, and S3A–S3C), which could be rescued with expression of exogenous SAMHD1-GFP (Figure 3B), suggesting that SAMHD1 may function in HR. To more directly determine if SAMHD1 functions in HR, we examined SAMHD1 depletion in U2OS cells integrated with a direct repeat (DR)-GFP reporter substrate in which expression of I-SceI endonuclease generates a DSB that when repaired by HR restores GFP expression (Pierce et al., 1999). SAMHD1 depletion caused an impairment in HR (Figure 3C), suggesting directly that SAMHD1 functions in HR. Notably, while SAMHD1 depletion in U2OS cells resulted...
in a 1.5- to 3-fold increase in dNTP pool concentration (Figures S3D and S3E), we did not observe any significant change in cell cycle (Figures 3D and 3E), suggesting that the observed effect is not due to an indirect effect of cell cycle change. Consistent with this finding, SAMHD1 depletion in HeLa cells and non-tumorigenic BEAS-2B cells impaired RAD51, but not γH2AX foci accumulation in response to CPT and IR treatment (Figures S3F–S3H, S3F, and S3G), indicating that SAMHD1 is required for HR, but not for DSB induction by CPT. In contrast, SAMHD1 depletion in U2OS cells transfected with the pEGFP-Pem1-Ad2 NHEJ reporter substrate (Seluanov et al., 2004) caused no significant impairment in NHEJ and only a mild increase in NHEJ with one siRNA (Figures S3H and S3I), implying that SAMHD1 specifically promotes HR, but not NHEJ in DSB repair.
HR is initiated by DNA end resection. Thus, we examined for RPA32 phosphorylation at Ser4/8, a marker for DNA end resection following CPT treatment. SAMHD1 depletion in U2OS cells impaired RPA32 Ser4/8 phosphorylation, but not total RPA32 levels in response to CPT (Figure 4A). Consistent with these findings, SAMHD1 depletion and KO in U2OS, BEAS-2B, and primary small airway epithelial cells caused a significant decrease in RPA70 foci formation in response to CPT and IR (Figures 4B, 4C, S4A–S4D, and S4F–S4I) and moreover impaired GFP-RPA70 recruitment to DNA damage sites induced by laser microirradiation (Figures 4D and S4E). ATRIP localization to DNA damage sites is dependent on its interaction with RPA-ssDNA. In

**Figure 2. SAMHD1 Localizes to DSBs in Response to DNA Damage**

(A–C) HeLa cells were treated with 2 μM CPT for 4 hr, fixed, and processed for immunofluorescence with indicated antibodies.

(A) Percent cells with SAMHD1 foci are shown.

(B and C) Representative immunofluorescence images of SAMHD1 co-localizing with γH2AX (B) or RAD51 (C) after DNA damage are shown.

(D) U2OS cells expressing SAMHD1-GFP were microirradiated, fixed after 1 min, and processed for immunofluorescence with anti-RPA70 antibodies.

(E and F) U2OS cells were treated with 0.1 μM CPT and 10 μM EdU for 1 hr, washed, and processed 1 hr for immunofluorescence with click chemistry and anti-SAMHD1 and CtIP antibodies. Quantitation (E) and representative SR images (F) of co-localization between nascent DNA (naDNA via EdU) and SAMHD1 or CtIP showing increased association upon CPT damage are shown.

(A and E) Mean and SEM from at least three independent replicas are shown. ***p < 0.001 and ****p < 0.0001. See also Figure S2.
In this regard, SAMHD1 depletion in cells also impaired GFP-ATRIP foci accumulation in response to CPT (Figures 4E and 4F). Moreover, SAMHD1 depletion in cells impaired CPT-induced ATR autophosphorylation at Thr-1989, but not total ATR levels (Figure 4G), suggesting that SAMHD1 is required for efficient ATR activation following CPT treatment. To more directly determine the role of SAMHD1 in DNA end resection, we labeled U2OS cells with BrdU, treated the cells with CPT, and probed the cells for BrdU exposure under non-denaturing conditions, which labels ssDNA. SAMHD1 depletion also impaired BrdU foci under these conditions (Figures 4H and 4I), providing direct support for SAMHD1 in promoting DNA end resection. Importantly, given its role as a dNTPase, SAMHD1 depletion in these cells caused only a mild, but insignificant, impairment in BrdU incorporation under denaturing conditions (Figure S4J) and to a much lesser extent than impairment in CPT-induced BrdU foci (cf. Figure 4I). Taken together, these data strongly suggest that SAMHD1 facilitates HR and ATR activation by promoting DNA end resection.

**SAMHD1 Promotes HR and DNA End Resection Independent of Its dNTPase Activity**

SAMHD1 has been proposed to maintain genome integrity by regulating dNTP pools. To determine if SAMHD1 dNTPase activity is required for DSB repair, we performed rescue experiments with SAMHD1-RFP H206A/D207A, which disrupts SAMHD1’s active site and impairs its dNTPase activity (Goldstone et al., 2011; Laguette et al., 2011) and reported DNase/RNase activities (Beloglazova et al., 2013). Both SAMHD1-RFP wild-type...
(WT) and H206A/D207A restored the HR impairment of SAMHD1 depletion in U2OS cells (Figures 5A, 5B, and S5A), suggesting that SAMHD1’s role in promoting HR is independent of its dNTPase activity. SAMHD1-HA WT and H206A/D207A also alleviated the IR-induced RPA70 foci impairment of SAMHD1 depletion in U2OS cells (Figure 5C), suggesting that SAMHD1 promotes DNA end resection independent of its dNTPase activity.

**SAMHD1 Interacts in a Complex with CtIP in Response to DNA Damage**

Because our findings suggest that SAMHD1 facilitates HR by promoting DNA end resection and its role in HR is independent of its active-site catalytic activity, we determined whether SAMHD1 might function with other nucleases. Co-immunoprecipitation (coIP) of SAMHD1-HA expressed in 293T cells pulled down GFP-CtIP and endogenous MRE11 (Figure 5D), and, similarly, coIP of CtIP-FLAG pulled down SAMHD1-RFP in response to IR (Figure 5E), suggesting that SAMHD1 interacts with CtIP and MRE11 in a damage-regulated manner. We validated that endogenous SAMHD1 coIPs with endogenous CtIP in response to IR (Figure 5F), and that endogenous SAMHD1 coIPs with GFP-MRE11 in response to IR (Figure S5B). The coIP of endogenous SAMHD1 with CtIP and MRE11 following IR was preserved even following benzamide nuclelease treatment (Figure S5C), suggesting that the damage-regulated interaction of SAMHD1 with CtIP and MRE11 is not mediated through DNA. Moreover, bacterially recombinant SAMHD1 pulled down recombinant GST-CtIP (Figure 5G), suggesting that SAMHD1 binds directly to CtIP. To identify the region of SAMHD1 that interacts with CtIP, we generated SAMHD1 deletion mutants and performed coIP of SAMHD1 WT and mutants with GFP-CtIP expressed in 293T cells. In contrast to SAMHD1-HA (1–115), SAMHD1-HA (115–562) coIP’d with GFP-CtIP (Figures 5H and 5J), suggesting that the HD, but not SAM, domain region of SAMHD1 is sufficient for CtIP interaction. In further mapping experiments, SAMHD1-HA (1–562), but not SAMHD1 (1–465), coIP’d with GFP-CtIP expressed in 293T cells (Figures 5I and 5J), suggesting that SAMHD1 amino acids (aa) 465–562 are necessary for interaction with CtIP. To provide insight into the binding surface of the CtIP interaction domain of SAMHD1, we examined the crystal structure of tetrameric SAMHD1 (Ji et al., 2013) and observed that aa 465–562 are located on the surface of tetrameric SAMHD1 (Figure S5D). Interestingly, a naturally occurring cancer-associated SAMHD1 mutation (K484T) from a patient with gastric cancer reported in The Cancer Genome Atlas (TCGA) through the cBioPortal for Cancer Genomics (Cerami et al., 2012) is located in this region facing the outside of tetrameric SAMHD1 (Figure S5D) and is evolutionarily conserved (Figure 5J). While SAMHD1-GFP K484T overexpressed in cells showed no significant impairment in dNTPase activity compared with SAMHD1-GFP WT (Figures S5E and S5F), SAMHD1-HA K484T showed an impairment in coIP with GFP-CtIP compared with SAMHD1-HA WT (Figure 5K), suggesting that K484 is critical for SAMHD1’s interaction with CtIP, but not dNTPase activity, and that a cancer-associated SAMHD1 mutation is functionally significant in impairing the interaction of SAMHD1 with CtIP.

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SAMHD1 has been reported to possess nuclease activity (Belogazlova et al., 2013; Ryoo et al., 2014), which has since been attributed to persistent co-purifying contaminants (Antonacci et al., 2016; Seamon et al., 2015). Our finding of a rescue of the HR and DNA end resection impairment of SAMHD1 depletion with an active-site mutant of SAMHD1, which abolishes its dNTPase activity, supports a model in which SAMHD1 promotes DSB repair by HR independent of its well-established dNTPase activity by facilitating CtIP recruitment, which in turn cooperates with MRN to promote DNA end resection (Figure 7F).

**DISCUSSION**

Our findings reveal a dNTPase-independent function for SAMHD1 in promoting HR-mediated DSB repair by facilitating DNA end resection through CtIP accrual, demonstrating that SAMHD1 has a direct role in genome maintenance independent of its role in dNTP pool regulation, establishing SAMHD1 as a critical regulator of DNA end resection in promoting DSB repair by HR and identifying the CtIP/MRE11 nuclease as unique interacting partners for SAMHD1. In this regard, we found that SAMHD1 deficiency by genetic knockdown or KO or proteasomal degradation by VLPs containing Vpx in cells causes IR, CtIP, and etoposide hypersensitivity, and SAMHD1 is recruited to DSBs that co-localize with γH2AX, RAD51, RPA70, and naDNA in response to DNA damage. SAMHD1 depletion further causes PARP inhibitor sensitivity, impaired RAD51 recruitment to foci, and impaired HR, but not NHEJ, through direct reporter assays. Moreover, SAMHD1 depletion impairs BrdU exposure, RPA Ser4/8 phosphorylation, and RPA recruitment to DSBs, suggesting that SAMDH1 facilitates DNA end resection. SAMHD1 depletion also impairs CPT-induced ATRIP foci accumulation and ATR autophosphorylation, suggesting that SAMHD1 is required for efficient ATR activation following CPT treatment. Mechanistically, SAMHD1 interacts directly with CtIP via an evolutionarily conserved domain in its C terminus, which is disrupted by a naturally occurring cancer-associated SAMHD1 mutation, and MRE11 in a damage-regulated manner, and is required for CtIP, but not MRE11, recruitment to DNA damage sites. In contrast to dNTPase-inactive SAMHD1, which is proficient for HR and DNA end resection, the cancer-associated SAMHD1 mutant with impaired CtIP interaction and proficient dNTPase activity fails to rescue the damage-induced CtIP recruitment deficit, IR-induced RPA70 foci impairment, and CPT hypersensitivity of SAMHD1 deficiency. Thus, our findings support a model in which SAMHD1 promotes DSB repair by HR independent of its well-established dNTPase activity by facilitating CtIP recruitment, which in turn cooperates with MRN to promote DNA end resection (Figure 7F).

**Figure 5. SAMHD1 Promotes HR and DNA End Recessed Independent of Its dNTPase Activity and Complexes with CtIP in Response to DNA Damage**

(A) U2OS cells integrated with a DR-GFP HR reporter substrate were transfected with indicated siRNAs, cDNAs, and I-Scel and fixed. GFP-positive cells were gated and analyzed for HR by GFP expression using flow cytometry.

(B) Western blot analysis in U2OS cells demonstrating SAMHD1 knockdown and expression of SAMHD1-RFP.

(C) U2OS cells were transfected with indicated siRNAs and cDNA, treated with 10 Gy IR, and processed 4 hr later for immunofluorescence with indicated antibodies. Quantitation of percent γH2AX-positive cells with RPA70 foci that are HA-SAMHD1 positive for complemented cells is shown.

(D) 293T cells were transfected with GFP-CtIP and SAMHD1-HA, treated with 10 Gy IR, harvested 4 hr later, IP’d with anti-HA antibodies, run on SDS-PAGE, and immunoblotted with indicated antibodies.

(E) 293T cells were transfected with CtIP-FLAG and SAMHD1-RFP, treated with 10 Gy IR, harvested 4 hr later, IP’d with anti-FLAG antibodies, run on SDS-PAGE, and immunoblotted with indicated antibodies.

(F) Endogenous SAMHD1 was IP’d from lysate from HCT-116 cells treated with or without IR, washed, separated by SDS-PAGE, and immunoblotted with indicated antibodies.

(G) Recombinant GST-CtIP and SAMHD1 purified from E. coli was pulled down with an anti-SAMHD1 antibody, washed, separated by SDS-PAGE, and immunoblotted with indicated antibodies.

(H and I) 293T cells were transfected with full-length or truncated SAMHD1-HA WT and GFP-CtIP, treated with 10 Gy, IR, harvested 4 hr later, IP’d with anti-HA antibodies, run on SDS-PAGE, and immunoblotted with indicated antibodies. Domain mapping analysis indicates that aa 115–562 are sufficient for interaction with CtIP (H) and aa 465–562 are necessary for interaction with CtIP (I).

(J) Schematic representation of SAMHD1 structural domains and evolutionary conservation of CtIP interaction domain.

(K) A naturally occurring cancer-associated SAMHD1 mutation (K484T) impairs the interaction of SAMHD1 with CtIP.

(A and C) Mean and SEM from three independent replicas are shown. **p < 0.01 and ***p < 0.001. See also Figure S5.
Figure 6. SAMHD1 Recruits CtIP to DNA Damage Sites and Chromatin in Response to DNA Damage and Promotes DNA End Resection through Its Interaction with CtIP

(A and B) U2OS cells were treated with 0.1 μM CPT and 10 μM EdU for 1 hr, washed, and processed 1 hr for immunofluorescence with click chemistry and anti-SAMHD1 and CtIP antibodies.

(A) Quantitation of co-localization of SAMHD1 and CtIP upon CPT damage.

(legend continued on next page)
suggest that SAMHD1’s role in promoting DNA end resection is likely independent of any intrinsic catalytic activity and thus through a scaffold function. In this respect, SAMHD1 localizes to naDNA at CPT-induced DSBs and binds to ssDNA/RNA (Beloglazova et al., 2013; Goncalves et al., 2012; Seamon et al., 2015, 2016; Tüngier et al., 2013) and could facilitate CtIP recruitment to or stabilization at DSBs through this interaction. CtIP recruitment to DNA damage sites is also dependent on its interaction with the MRN complex (You et al., 2009; Yuan and Chen, 2009), BRCA1 (Yu et al., 2006), and its own tetramerization (Wang et al., 2012), which could be regulated by SAMHD1.

Given SAMHD1’s well-established role as a dNTPase, how might this activity be reconciled with its role in promoting DNA end resection and HR? SAMHD1 binds to ssDNA at its dimer-dimer interface, which sterically blocks its tetramerization into its dNTPase active form (Brandariz-Nuñez et al., 2013; Hansen et al., 2014; Ji et al., 2014; Seamon et al., 2016; Yan et al., 2013; Zhu et al., 2013). Thus, SAMHD1 may function as a monomer or dimer in promoting DNA end resection and tetramer in promoting dNTP metabolism, which is controlled by its binding to ssDNA in response to DNA DSB induction. This may explain, at least in part, why SAMHD1 overexpression, which may facilitate its tetramerization, does not fully rescue the CPT hypersensitivity of SAMHD1 deficiency. Our finding that SAMHD1 promotes DNA end resection provides further support for a common role in nucleic acid metabolism that is shared by SAMHD1 and other AGS susceptibility proteins TREX1, RNaseH2, and ADAR1, suggesting that SAMHD1’s role in DNA DSB repair may be important for preventing improper innate immune response and autoimmune disease.

A role for SAMHD1 in maintaining genome integrity and preventing cancer has previously been attributed to its activity in dNTP pool regulation (Clifford et al., 2014; Franzolin et al., 2013; Kohnen et al., 2015; Kretschmer et al., 2015; Rentoft et al., 2016). Our findings now demonstrate that SAMHD1 also has a direct role in genome maintenance by promoting DNA end resection to facilitate DSB repair by HR independent of its canonical role in dNTP metabolism. Given that SAMHD1 is dysregulated in a number of cancers, SAMHD1’s role in DSB repair may help explain, at least in part, how its dysregulation is associated with genomic instability and carcinogenesis. Indeed, our findings show that a naturally occurring gastric cancer-associated SAMHD1 mutation impairs the interaction of SAMHD1 with CtIP, but not its dNTPase activity, and the mutant SAMHD1 fails to rescue the CtIP recruitment deficit, DNA end resection impairment, and CPT hypersensitivity of SAMHD1 deficiency, suggesting that SAMHD1’s interaction with CtIP may be important for the prevention of genomic instability and cancer. As several heterozygous colorectal cancer-associated mutations impair SAMHD1’s dNTPase activity, and increased dNTP levels contribute to mutagenesis (Rentoft et al., 2016), collectively, these findings support a role for SAMHD1 in maintaining genome integrity and preventing cancer through dual roles in DNA end resection and dNTP metabolism.

As our data suggest that SAMHD1 plays a critical role in the response of cancer cells to DSB-inducing agents, SAMHD1 may also be a promising therapeutic target for cancer therapies that induce DSBs. Our finding that targeting SAMHD1 for proteasomal degradation with VLPs containing Vpx sensitizes cancer cells to DSB-inducing agents provides rationale for the use of VLPs containing Vpx in augmenting the efficacy of IR, PARP inhibitor, and other DSB-inducing agents as a potential approach for cancer therapy. Another rationale-driven approach for cancer therapy based on our work will be to disrupt the interaction of SAMHD1 and CtIP with small molecule inhibitors to be used as an adjunct to DSB-inducing agents.

EXPERIMENTAL PROCEDURES

Cell Culture, Plasmids, and siRNA

293T, HeLa, HCT-116, MCF7, and U2OS cells were grown in DMEM (GIBCO), supplemented with 7.5% fetal bovine serum (FBS). Stably transfected cells were maintained with 1 μg/mL puromycin (Fisher). SAMHD1-GFP/RFP plasmids were generated by cloning SAMHD1 in pcDNA3.1-GFP/RFP (Addgene, # 70219 or 13032, respectively) using EcoRI/BamHI restriction sites. SAMHD1 WT and truncations were cloned in pKH3 (Addgene, # 12555) using EagI and XbaI to generate SAMHD1-HA. GFP-CtIP, FLAG-CtIP, and GFP-FLAG-MRE11 plasmids were obtained from Dr. Steve Jackson (Sartori et al., 2007; Schmidt et al., 2015). GFP-RPA70 plasmid was obtained from Dr. Marc Wold (Haring et al., 2008). ATRIP-GFP plasmid was obtained from Dr. Akira Matsusuka (Itakura et al., 2005). Plasmid transfections were performed using Lipofectamine 2000 (Invitrogen), following manufacturer’s instruction. Cells

(B) Representative SR images of a single foci showing SAMHD1/CtIP co-localization in cells also labeled for naDNA.
(C) U2OS cells integrated with a DR-GFP HR reporter substrate were transfected with indicated siRNAs and I-SceI, fixed, and analyzed for HR by GFP expression using flow cytometry.
(D) U2OS cells transfected with CtIP, SAMHD1, or NT siRNA were treated with 2 μM CPT for 4 hr, fixed, and processed for immunofluorescence with indicated antibodies. Quantitation of relative percent γH2AX-positive cells with RPA70 foci is shown.
(E) Representative images of GFP-CtIP-expressing U2OS cells transfected with NT or SAMHD1 siRNA, subjected to laser microirradiation, fixed 5 min after damage, and processed for immunofluorescence with anti-γH2AX antibodies.
(F) HCT-116 cells transfected with SAMHD1 or NT siRNA were treated with 10 Gy IR and harvested 1 hr later for biochemical fractionation. Chromatin bound proteins were run on SDS-PAGE and immunoblotted with indicated antibodies. NS indicates non-specific band as loading control.
(G) Representative images of U2OS SAMHD1 WT and KO cells expressing RFP-SAMHD1 and GFP-CtIP, subjected to laser microirradiation, fixed 5 min after damage, and processed for immunofluorescence with anti-γH2AX antibodies.
(H) U2OS cells were transfected with indicated siRNAs and cDNA, treated with 10 Gy IR, and processed 4 hr later for indirect immunofluorescence with anti-RPA70, HA, and γH2AX antibodies. Quantitation of percent γH2AX-positive cells with RPA70 foci that are SAMHD1-GFP-positive for complemented cells, from three independent replicates of 60 cells counted each is shown.
(I) U2OS cells transfected with indicated siRNAs and plasmids were treated with 200 nM CPT for 72 hr prior to assaying for viability with Alamar Blue. The treated to untreated viability relative to NT siRNA is shown.
(J) Western blot analysis showing expression of SAMHD1-GFP and endogenous SAMHD1 in U2OS cells 72 hr after siRNA transfection and 48 hr after cDNA transfection. The higher levels of SAMHD1 in the SAMHD1-GFP-transfected cells likely represent exogenous SAMHD1 cleaved from SAMHD-GFP.
were transfected with siRNA using Lipofectamine RNAi Max (Invitrogen), following the manufacturer’s instructions. siRNAs were purchased from Dharmacon or QIAGEN.

**Immunoblot**

Cells were harvested in PBS and lysed for 30 min on ice in IP lysis buffer (200 mM NaCl, 0.75% Chaps, and 50 mM Tris pH 8.0) or radioimmunoprecipitation assay (RIPA) buffer (10 mM Tris-Cl [pH 8.0], 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, and 140 mM NaCl) (Hall et al., 2014), supplemented with protease inhibitors. Lysates were clarified by centrifugation (13,000 rpm for 10 min at 4°C) and the supernatants were collected. Protein samples were quantified with the Bradford assay and resolved by SDS-PAGE, transferred onto polyvinylidene fluoride (PVDF), and probed using the indicated primary antibodies. The membrane was stained with Alexa Fluor 680 or 800 anti-mouse/rabbit secondary antibody (Life Technologies) and visualized with the LI-COR Odyssey system. The following antibodies were used for staining: GAPDH (Santa Cruz, sc-47724); FLAG (Santa Cruz, sc-51590); GFP (Abcam, Ab6558); HA (Sigma, H9658), RPA70 (Cell Signal, 2267), RPA32 (Santa Cruz, sc-14692), SAMHD1 (OriGene, TA502024), CtIP (14-1, a generous gift from Richard Baer) (Yu and Baer, 2000), BRCA2, RAD51 (Calbiochem, PC130), γH2AX (Cell Signal, 2577 or Millipore, 05-636), 53BP1 (Bethyl Labs, A300-273A), BrdU (BD Biosciences, 347580), and MRE11 (Abcam, ab30725).

**Cell Viability Assay**

Cell viability assays were performed as previously described (Colbert et al., 2014; Smith et al., 2014; Yu et al., 2010; Zhang et al., 2013). Briefly, cells were plated at a density of 5 x 10^6 cells/well in 6-well plates and siRNA transfected at 25 nM. 24 hr post knockdown, media were replaced with or without overexpression solution containing media supplemented with plasmid DNA, Lipofectamine 2000, and Opti-MEM solution. Cells were treated with VLP containing Vpx or no Vpx, as described previously (Berger et al., 2011; Kim et al., 2012), with minor modification. 24 hr after initial transfection or transduction with Vpx VLPs, cells were trypsinized, counted, and replated in triplicate to a
density of 2 x 10^5 cells/well in 96-well plates. 24 hr after plating, cells were treated with media containing the drug for 72 hr. Cell viability was then assessed via Alamar Blue (resazurin) reagent, incubated at 1:10 dilution for 6 hr, and assayed for fluorescence according to the manufacturer’s protocol. Viability fractions were normalized to vehicle-treated controls exposed to identical transfection conditions.

Statistical Analysis
Unless otherwise stated, experiments were performed at least three times and analyzed using unpaired two-tailed Student’s t test and data expressed as mean ± SEM, p < 0.05 was considered statistically significant.


Details of these methodologies can be found in Supplemental Experimental Procedures.

ACCESSION NUMBERS
The accession number for SAMHD1 crystal structure used to generate a Figure SSD is RCSB PDB: 4BZB.

SUPPLEMENTAL INFORMATION
Supplemental Information includes Supplemental Experimental Procedures and seven figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2017.08.008.

AUTHOR CONTRIBUTIONS

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