Structure and function of an effector domain in antiviral factors and tumor suppressors SAMD9 and SAMD9L

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Edited by Ian Wilson, Integrative Structural and Computational Biology, Scripps Research Institute, La Jolla, CA; received September 8, 2021; accepted December 8, 2021

SAMD9 and SAMD9L (SAMD9/9L) are antiviral factors and tumor suppressors, playing a critical role in innate immune defense against poxviruses and the development of myeloid tumors. SAMD9/9L mutations with a gain-of-function (GoF) in inhibiting cell growth cause multisystem developmental disorders including many pediatric myelodysplastic syndromes. Predicted to be multidomain proteins with an architecture like that of the NOD-like receptors, SAMD9/9L molecular functions and domain structures are largely unknown. Here, we identified a SAMD9/9L effector domain that functions by binding to double-stranded nucleic acids (dsNA) and determined the crystal structure of the domain in complex with DNA. Aided with precise mutations that differentially perturb dsNA binding, we demonstrated that the antiviral and antiproliferative functions of the wild-type and GoF SAMD9/9L variants rely on dsNA binding by the effector domain. Furthermore, we showed that GoF variants inhibit global protein synthesis, reduce translation elongation, and induce proteotoxic stress response, which all require dsNA binding by the effector domain. The identification of the structure and function of a SAMD9/9L effector domain provides a therapeutic target for SAMD9/9L-associated human diseases.

SAMD9/9L have also been implicated in antiviral defense against several viruses, most prominently poxviruses (12–14). SAMD9/9L are ubiquitously expressed in many tissues (15), and their expression can be further induced by interferons. Nearly all mammalian poxviruses encode at least one specific inhibitor against SAMD9/9L, with the prototypical poxvirus vaccinia encoding two inhibitors that target different regions of SAMD9/9L (16, 17). The deletion of both SAMD9/9L inhibitors, K1 and C7, from vaccinia virus resulted in abortive replication in most mammalian cells due to a shut-off of global protein synthesis (18–20).

SAMD9/9L are large cytoplasmic proteins with more than 1,500 amino acids (aa). They are predicted to contain multiple domains with an architecture reminiscent of Apaf-1 and NOD-like receptors (21), which are self-regulated signaling molecules containing separate sensor and effector domains. However, the molecular and structural basis for the diverse functions of SAMD9/9L are unknown. Here, we identified a 230-aa effector domain in SAMD9 that binds preferentially to double-stranded (ds) nucleic acids (dsNA) and determined its crystal structure in complex with DNA. Utilizing precise mutations that disrupt

Significance

SAMD9 and SAMD9L (SAMD9/9L) are important innate immune defenders against viruses and the development of myeloid tumors. They form a crucial host barrier that poxviruses must overcome for successful infection. A myriad of human diseases including many pediatric myelodysplastic syndromes are caused by mutations in SAMD9/9L. However, the molecular functions of SAMD9/9L and how their functions are executed are unknown, hindering progress in developing effective therapies for SAMD9/9L-associated human diseases. Here, we identified the structure and function of a SAMD9/9L effector domain that is essential for their physiological functions as well as the pathogenic effects exerted by patient-derived mutations. Our study revealed a potential therapeutic target for SAMD9/9L-associated human diseases.

Author contributions: J.D. and Y.X. designed research; S.P., X.M., F.Z., P.K.P., J. Chaturvedi, J. Coronado, and M.M. performed research; Y.M., S.-B.Q., J.D., and Y.X. analyzed data; and J.D. and Y.X. wrote the paper.

The authors declare no competing interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at http://www.pnas.org/lookup/suppl;doi:10.1073/pnas.2116550119/-/DCSupplemental.

PNAS 2022 Vol. 119 No. 4 e2116550119
https://doi.org/10.1073/pnas.2116550119 | 1 of 9
dsDNA binding to different degrees, we demonstrated that dsDNA binding is essential for the seemingly diverse functions of SAMD9/9L in tumor suppression, antiviral responses, and translation control.

Results
Identification of a dsDNA Binding Domain in SAMD9. SAMD9 aa 195 to 352 bears some sequence similarity to the NA-binding Alba domain (21). Based on our secondary structure analysis, a larger SAMD9 region of aa 134 to 385 (SAMD9134-385) was expressed and purified to assess its binding to NA with the electrophoresis mobility shift assay (EMSA). SAMD9134-385 formed stable complexes with synthetic dsRNA and dsDNA of various lengths from 18- to 30-nucleotide (nt) but not with an 11-nt dsDNA (SI Appendix, Fig. S1A). Complex optimal formation appeared to be achieved when the protein and DNA were mixed at 2:1 molar ratio (SI Appendix, Fig. S1B). The binding of SAMD9134-385 with a fluorescence-labeled dsDNA can be effectively competed off with unlabeled dsDNA, dsRNA, and poly IC but only slightly with single-stranded (ss) RNA and ssDNA (SI Appendix, Fig. S1C), indicating that SAMD9134-385 preferentially binds dsDNA.

The Crystal Structure of SAMD9156-385 in Complex with DNA. Through limited proteolysis of SAMD9134-385, a smaller domain that retained the dsDNA binding activity was identified (SI Appendix, Fig. S2 A and B). The compact domain of SAMD9156-385 can be crystallized with a 2.2-nt dsDNA, and the complex crystal structure was determined at 2.8-Å resolution. Although purified as a monomer, the protein DNA complex does not have any close match when searched using the Secondary Structure Matching (SSM) server (23). The overall structure does not resemble that of a canonical B-form dsDNA helix (Fig. 1 A and B). The two protein protomers are related by a noncrystallographic twofold symmetry that is perpendicular to the DNA axis. One protomer is shifted along the DNA axis by 8 nt with respect to the other (Fig. 1B). Together, the dimer covers 22 nt of DNA in length with nearly no protein–protein contacts.

The structures of the two protomers closely resemble each other with an RMSD of 0.42 Å over 207 aligned residues. The structure is compact and mainly comprises six α-helices and 14 β-strands with an overall dimension of ~53 x 33 x 30 Å. Most of the β-strands are short with 2 to 3 aa except for β-4, β-5, β-9, and β-10, which form a central β-sheet together with β-8, packed by five short strands (β-1, β-2, β-3, β-6, and β-7) on one side and by three α-helices (α-1, α-2 and α-3) on the opposite side. The 10 β-strands together form an asymmetrical β-barrel. Two short β-hairpins (β-11 to β-12 and β-13 to β-14) and three additional helices (α-4, α-5, and α-6) are present at the C terminus (Fig. 1C). The loops between β-3 and β-4, β-9 and β-10, and β-11 and β-12 are disordered. The central β-sheet and helices α-1 and α-2 resemble an Alba domain fold (22), but the overall structure does not have any close match when searched using the Secondary Structure Matching (SSM) server (23).

SAMD9156-385 mainly contacts the phosphate backbone of the DNA without interacting with any DNA bases, suggesting a sequence-independent binding mode. In both protomers, charge–charge interactions with the DNA cluster at three basic residues, K198 located on the loop β-4 to α-1 and K214 and R221 located on helix α-1. In protomer A, K198 forms a salt bridge with the nonbridging oxygen of the backbone phosphate at the nucleotides T7 to A8. K214 contacts the DNA backbone at the nucleotides A8 to A9. R221 contacts the nucleotides T11 to C12 in the other DNA strand (Fig. 1D). In protomer B, both K198 and K214 contact the DNA backbone at the T7 to A8 in the other strand, while R221 contacts the DNA backbone at the A12 to T13. Additional contacts between the protein and the backbone of the DNA are observed from the side chains of K242 on loop β-6 to β-7 and K350 on strand β-14. These five basic residues are also highly conserved among SAMD9/9L family members (SI Appendix, Fig. S3A). Human SAMD9 and SAM9L share ~60% aa identity, and a model structure of the corresponding domain of SAMD9L was established based on this homology (SI Appendix, Fig. S3B). We hereafter refer to SAMD9156-385 and the corresponding region in the SAMD9/9L family as the dsNA-binding domain (DBD).

Mechanism of DNA Binding by SAMD9 DBD. We selected the five conserved basic residues observed to contact DNA (K198, K214, R221, K242, and K350) and an additional conserved basic residue (K257) far away from the DNA interface for mutagenesis studies. The residues were individually substituted with glutamate to reverse the charge of the side chains. DNA binding by SAMD9154-385 in EMSA was not affected by K257E substitution, while it was completely disrupted by the K198E, K214E, and R221E substitutions and partially disrupted by the K242E and K350E substitutions, indicating that residues K198, K214, and R221 each play an essential role, while K242 and K350 are only partially required for DNA binding (Fig. 1E). The binding affinity of SAMD9134-385 with the 22-nt dsDNA was determined to be ~16 µM by the fluorescence polarization assay (Fig. 1F). The binding affinity was similarly affected by the substitutions as in EMSA, except that the K214E mutant showed a low but better than expected binding affinity (~222 µM).

DBD dsNA Binding Activity is Essential for the Antiviral Effect of the Wild-type (WT) and GoF SAMD9/9L Variants. Having defined a set of DBD mutations that reduce dsNA binding to different levels, we next utilized these mutations to assess the importance of dsNA binding in SAMD9/9L functions. We first studied the effects of the DBD mutations on the antiviral activities. A vaccinia virus mutant deleted of the viral SAMD9/9L inhibitors (vK1′ C7/GFP+) would replicate and express the green fluorescent protein (GFP) reporter only in cells that have no or low SAMD9/9L expression, including the human breast cancer BT20 and HEK 293T cells (12, 17). In BT20 cells that were transduced with a doxycycline-regulated SAMD9 transgene, productive viral replication rate (GFP+%) was reduced by ~50% after the transgene was specifically induced (SI Appendix, Fig. S4A). By contrast, productive replication was reduced by ~30% by the K242E mutant and not affected by the K214E and R221E mutants, correlating with the effects of the mutations on dsNA binding. Although the K257E mutant had no defect in dsNA binding, it was also defective at inhibiting viral replication, indicating that the conservative K257 residue plays a separate but essential role for SAMD9 function. To further evaluate the mutants’ effect on viral replication, we also performed the traditional viral growth assay. The yield of vK1′ C7 at 24 h postinfection was reduced by ~10-fold in BT20 cells that were induced to express the WT or K242E mutant, while it was not reduced in cells expressing similar levels of the K214, K221E, or K257E mutants (SI Appendix, Fig. S4B). A mock-transduced antiviral effect was observed in clonally selected SAMD9-expressing cells, where the 24-h yield of vK1′ C7 was reduced by more than 100-fold by the WT SAMD9 while not reduced by the K214E or R221E mutants (SI Appendix, Fig. S4B), confirming that the K214E and R221E mutations abolish the antiviral activity.

To assess antiviral activities of a wide variety of SAMD9/9L mutants at single-cell level (Fig. 2A), mCherry-SAMD9/9L fusion was transfected into 293T cells for 36 h followed by infection with vK1′ C7 for 15 h. vK1′ C7 infection rate (GFP+%) in cells expressing different levels of SAMD9/9L (mCherry+).
was quantified by flow cytometry (Fig. 2B and C). Nontransfected cells (mCherry−) from the same culture well served as the internal control. When substantial infection occurred in transfected cells, viral replication level as indicated by GFP intensity was also quantified (Fig. 2D). The infection rate and replication level were not reduced by the DBD-defective K198E, K214E, and R221E mutants, while they were reduced to different levels by the DBD partially defective K242E and K350E mutants (Fig. 2E and F). Notably, the DBD-defective mutants failed to inhibit viral replication irrespective of their expression levels, but the cellular levels of the K242E and K350E mutants had a dose-dependent effect on their antiviral activities (Fig. 2C). More specifically, viral replication became nonpermissible only in cells that expressed a high level of the K350E mutant or an intermediate to high level of the K242E mutant (Fig. 2C, Upper Right quadrant), indicating that the partial defect of the two mutants can be overcome with overexpression. Overall, the effects of the DBD mutations on antiviral activities are similar in magnitude to their effects on dsDNA binding. The K257E mutant did not reduce the infection rate but decreased the viral replication level (Fig. 2D and F), indicating that its antiviral activity is only partially defective, but the partial defect cannot be overcome with overexpression as in the case of K242E and K350E.
To assess the relationship between the antiproliferative and antiviral activities, we next tested various patient-derived GoF SAMD9/9L variants (Fig. 2A), including R982C, I983S, E1136Q, and R1281K of SAMD9, and H880Q, W1180R, R1281K, and F886Lfs*11 of SAMD9L. They are representative of the pathogenic mutations identified in human patients, which predominately occur at the C-terminal half, particularly the putative NTPase domain (Fig. 2A). All the GoF variants, including the frameshifted SAMD9L F886Lfs*11, displayed a similar inhibitory effect on vaccinia virus replication as the WT SAMD9/9L (Fig. 2E and F and SI Appendix, Fig. S5). Also similarly, the antiviral effect of the GoF variants can be abolished by introducing the DBD-defective, R221E or the equivalent R223E mutation to SAMD9 or SAMD9L, respectively (Fig. 2E and SI Appendix, Fig. S3). Altogether, our data showed that the DBD dsNA binding activity is critical for the antiviral effect of the WT and GoF SAMD9/9L variants.

**DBD dsNA Binding Activity Is Essential for the Antiproliferative Activities of the WT and GoF SAMD9/9L Variants.** To determine the impact of the DBD mutations on antiproliferative activities of SAMD9/9L, we transfected the mCherry-SAMD9/9L fusions into 293T cells and examined their effects on cell proliferation and cell cycle by quantifying the total as well as newly divided cell populations with the GFP MFI shown. Relative infection rates (mCherry+/mCherry-) among SAMD9/9L-expressing and nontransfected cells are derived from the flow cytometry data shown in both Fig. 2 and SI Appendix, Fig. S5. Each biological replicate and SD are shown. Statistics: one-way ANOVA compared to the WT or R221E (ns, not significant; ****P < 0.0001).
synthesized DNA in the cells (Fig. 3A and B). SAMD9L expression significantly reduced the number of proliferating, S-phase cells relative to that of the nontransfected cells from the same culture well (Fig. 3B), and the level of cellular DNA synthesis was reduced in a SAMD9L dose-dependent manner (Fig. 3A, Upper Right quadrant). SAMD9 had a similar effect, but a higher threshold of SAMD9 expression appeared to be required for reducing cellular DNA synthesis (SI Appendix, Fig. S6A). The GoF mutations enhanced the antiproliferative activity by varying degrees, with R982C of SAMD9 and the H880Q

![Figure 3](https://example.com/figure3.png)

**Fig. 3.** dsDNA binding by DBD is essential for antiproliferative activities of the WT and GoF SAMD9/9L variants. mCherry-SAMD9/9L mutants as illustrated in Fig. 2A were transfected into HEK 293T cells for 24 h, and the total and newly synthesized cellular DNA were labeled with FxCycle and EdU (for 2 h), respectively. Representative flow cytometry plots of cellular EdU and mCherry levels in the total cell population (A), or cellular EdU and FxCycle levels among mCherry-SAMD9L+ or mCherry− population. (B) Relative S-phase (C, Upper) and DNA synthesis levels (C, Lower) between SAMD9-expressing and nontransfected cells from the same culture wells are derived from the flow cytometry data shown in Fig. 3 and SI Appendix, Fig. S6. Each biological replicate and SD are shown. Statistics: one-way ANOVA compared to the WT (ns, not significant; **P < 0.01; ****P < 0.0001).
and F886Lfs*11 of SAMD9L demonstrating the strongest effect (Fig. 3C), nearly preventing cells from synthesis of DNA. The GoF variants reduced cellular DNA synthesis even at low expression levels, and the cell cycle stalled primarily at the G0/G1 phase (Fig. 3B and SI Appendix, Fig. S6).

Like their effects on antiviral activities, the DBD-defective K198E, K214E, and R221E mutations inactivated, while K242E, K257E, and K250E mutations reduced the antiproliferative activity of the WT SAMD9 (Fig. 3C and SI Appendix, Fig. S6B). A similar effect was observed when the DBD mutations were combined with SAMD9R221E or SAMD9R223E mutation (Fig. 3C and SI Appendix, Fig. S6).

**GoF SAMD9/9L Variants Inhibit Global Protein Synthesis and Induce Proteotoxic Stress Response.** A shut-off of cellular protein synthesis is associated with abortive viral replication caused by SAMD9 (19, 24), so we studied whether SAMD9/9L had a direct effect on cellular protein synthesis. Metabolic labeling with a methionine analog in SAMD9-transduced BT20 cells did not reveal any obvious effect of the WT SAMD9 on cellular protein synthesis. However, a GoF SAMD9 variant, R1293W, greatly reduced global protein synthesis after SAMD9 expression was specifically induced for more than 6 h (Fig. 4A and SI Appendix, Fig. S7A). Analysis of the total cellular RNA and the transcriptome showed that RNA integrity and transcription of the vast majority of the cellular genes were not affected by the GoF variant (Fig. 4B and SI Appendix, Fig. S7B and Dataset S1). A total of 43 genes were significantly induced (Fig. 4B and SI Appendix, Fig. S7C), and gene ontology analysis indicated that they belong to the pathways of unfolded protein response or the responses to various eIF2α kinases, indicating that the GoF variant caused a proteotoxic stress response.

To quantify the effect of additional SAMD9/9L variants on global protein synthesis, we transiently transfected the variants into 293T cells and fluorescently labeled nascent cellular proteins with a puromycin analog. Flow cytometry analysis showed that the WT SAMD9/9L did not reduce cellular protein synthesis until their cellular expression reached a very high level (Fig. 5A, Upper). By contrast, the GoF SAMD9/9L mutants reduced protein synthesis in nearly all transfected cells, and the level of reduction correlated with SAMD9/9L expression level (Fig. 5A and SI Appendix, Fig. S8). Moreover, the effects of the GoF variants on protein synthesis correlate with the strength of their antiproliferative activities, with SAMD9R982C, SAMD9L982C, and SAMD9L986E,11 displaying the strongest effect (Fig. 5 and SI Appendix, Fig. S8). Overall, the quantitative flow cytometry analysis indicated that the GoF variants as well as a high cellular level of the WT SAMD9/9L had an inhibitory effect on cellular protein synthesis.

**DBD dsRNA Binding Activity Is Essential for SAMD9/9L to Inhibit Global Protein Synthesis and Reduce Translation Elongation.** In contrast to the WT and GoF SAMD9/9L variants, the DBD-defective K198E, K214E, and R221E mutants as well as the K257E mutant had no inhibitory effect on cellular protein synthesis, while the DBD partially defective K242E and K350E mutants had a small inhibitory effect only when they were expressed at a very high level (Fig. 5B and SI Appendix, Fig. S8). More strikingly, the strong inhibitory effect of the GoF variants on cellular protein synthesis was abolished when they were combined with the DBD-defective SAMD9R221E or SAMD9R223E mutation, resulting in similar levels of cellular protein synthesis in all cells regardless of the SAMD9/9L expression levels (Fig. 5 and SI Appendix, Fig. S8). Similar rescuing effects could even be detected from the total cell population by Western blot of puromycin-labeled proteins (SI Appendix, Fig. S9).

The effects of the SAMD9/9L mutants on cellular protein synthesis were also evident from quantitative analysis of SAMD9/9L protein levels in transfected cells. Mean fluorescence intensity (MFI) of various mCherry-SAMD9/9L GoF variants in transfected cells was significantly lower than that of the WT, which in turn was greatly reduced compared to that of the DBD mutants (SI Appendix, Fig. S10), despite only a few nucleotides difference between the constructs. The DBD-defective K198E, K214E, and R221E mutants had the highest MFI, while K350E, K242E, and K257E mutants had the intermediate MFI (SI Appendix, Fig. S10), correlating with the effects of the mutations on antiproliferative activities. Combining the DBD-defective SAMD9R221E or SAMD9R223E mutation with the GoF variants greatly increased the protein level from that of the GoF variants (SI Appendix, Fig. S10B).

A recent study showed that some GoF SAMD9L variants inhibited translation elongation (6). To determine whether SAM9D has a similar effect, we performed a modified “SunRiSE” assay that measures ribosome run-off with flow cytometry at single-cell resolution (25). Compared to nontransfected cells in the same culture well, cells expressing SAMD9 were slightly slower in ribosome run-off after new translation initiation was blocked with an inhibitor, resulting in a smaller reduction in active translation at 30 s post the initiation block (Fig. 5C). Ribosome run-off was slowed even further in cells expressing SAMD9R1293W, while it was not affected by...
Fig. 5. dsDNA binding by DBD is essential for SAMD9/9L to inhibit global protein synthesis and reduce translation elongation. (A) HEK 293T cells were transfected with mCherry-SAMD9/9L mutants as illustrated in Fig. 2A and labeled with OPP for 30 min. Representative flow cytometry plots of OPP level relative to cellular mCherry level are shown. Background level of OPP staining in cells that were treated with cycloheximide (CHX) is shown as a control. (B) Nascent protein synthesis levels in SAMD9/9L-expressing cells relative to nontransfected cells from the same culture wells are derived from the flow cytometry data shown in Fig. 5 and SI Appendix, Fig. S8. Each replicate and SD are shown. Statistics: one-way ANOVA compared to the WT (****P < 0.0001). (C) Identically transfected cells were treated with harringtonine to block new translation initiation. Active protein synthesis level at the indicated times after harringtonine treatment was assessed by measuring OPP level as described in (A) and normalized to the level at time 0 (T0). Each data point represents the average and SD of duplicate samples.
The crystal structure of SAMD9-DBD:DNA complex revealed a compact domain that primarily engages the DNA backbone in a sequence-independent manner. Although an Alba domain was predicted for SAMD9, our study identified a domain that is more than 70 aa larger than the predicted Alba domain, with a more complex structure than the canonical β-α-β-α-β-β Alba fold (22). Furthermore, the DNA contact surface in the SAMD9-DBD:DNA structure is different from that of the existing Alba:DNA structures. In a structure of aarchaean chromatin protein Alba2 domain (PDB code: 3U6Y) (26), the key DNA-contacting residues are predominantly located on the loops and the tip of helix α-2, with no involvement from α-1. By contrast, two of the three key contacting residues in SAMD9 (K214 and R221) are located on helix α-1, and α-2 is not involved.

Although the solved structure is of a complex with DNA, our EMSA data showed that the recombinant DBD can also bind dsDNA with affinity in a model of the dsDNA tetraloop is relatively weak (K_D = 16 μM). Considering their cytoplasmic site of action and the recently reported interactions with ribosomal components (27), SAMD9/9L may bind to double-stranded regions of cytoplasmic RNA such as ribosomal RNA. Since the antiproliferative effect depends on the NA binding, DBD binding of NA in full-length SAMD9/9L is likely subject to tight regulation by other domains or cellular partners. Notably, SAMD9/9L also have a predicted protein deacetylase domain (SIR2) that may regulate NA binding by deacetylating lysine residues (21). What NA SAMD9/9L bind in cells and how the binding is regulated remain to be determined.

Guided by the complex structure, we were able to precisely perturb the dsDNA binding activity without affecting the overall structure, which allowed us to demonstrate that the dsDNA binding activity is essential for the known functions of SAMD9/9L. Significantly, the different effects the mutations have on SAMD9/9L functions largely correlate with their effects on DNA binding, indicating that the effector functions are closely linked to the dsNA binding activity. SAMD9/9L are large, multimeric proteins, so it would not be surprising if they had separate effector domains for their diverse functions in antiviral defense and tumor suppression. However, we showed that the same NA binding residues are essential for both the antiviral and antiproliferative functions, suggesting that the two functions share at least one common molecular mechanism that requires dsNA binding by DBD. As one mutation (K257E) in DBD was found to disrupt SAMD9 functions without affecting NA binding, dsNA binding by DBD is necessary but not sufficient for the SAMD9/9L functions.

What might be the common molecular process underlying the antiviral and antiproliferative functions of SAMD9/9L? While dysregulation of endocytosis has been the main mechanism proposed for the antiproliferative function (1, 11), protein synthesis shutoff was recognized as the hallmark for host restriction of vK1 C7 (19), long before SAMD9/9L were discovered to be the culprits. We thus have focused on translational control as the potential underlying mechanism and found that GoF SAMD9/9L variants profoundly inhibit global protein synthesis. Several papers recently reported a similar inhibitory effect of the GoF variants on cellular protein synthesis while our manuscript was being prepared (6, 7, 27). We showed further that the same DBD residues that are essential for the antiviral and antiproliferative activities are also critical for SAMD9/9L to inhibit protein synthesis, suggesting that translational control is an important underlying mechanism for SAMD9/9L functions. How SAMD9/9L inhibit protein synthesis is unclear, but we found that a GoF SAMD9 variant induces a proteotoxic stress response that resembles the closest with the response of EIF2AK1. While PKR (EIF2AK2) was previously shown to be dispensable for SAMD9 antiviral activities (24, 28), whether other eIF-2α kinases are involved in SAMD9/9L functions is yet to be determined. Beyond a potential effect on translation initiation possibly through modulating eIF-2α, SAMD9L was shown recently (6), and SAMD9 was shown here, to reduce translation elongation. The DBD activity is also required for this process, but the mechanism is unknown.

There is one notable difference in SAMD9/9L activities under noninfectious and infectious conditions. Under noninfectious condition, GoF variants are highly potent protein synthesis inhibitors at WT inhibiting cell growth and cellular protein synthesis. By contrast, the WT and GoF variants are equally effective at inhibiting vaccinia virus replication. Since the GoF variants inhibit cellular protein synthesis in uninfected cells, their antiviral effects could simply reflect a preexisting block in translation when the infection occurs. The WT on the other hand has a minor effect on cellular protein synthesis in uninfected cells, but it achieves a similar level of viral inhibition as the GoF variants, suggesting that the WT is not intrinsically less active than the GoF variants. Rather, the relative minor effect of the WT under noninfectious conditions is probably due to an autoinhibitory effect, which is relieved by viral infection. GoF mutations probably disrupt the autoinhibitory mechanism. Specifically, the C-terminal half that is truncated by the frameshift GoF SAMD9L variant is likely involved in the autoinhibition. Our findings that the dsNA binding residues are required even for the activities of the frameshift variant again illustrate that DBD is a key effector domain and its dsNA binding activity is an essential effector function.

Materials and Methods
Recombinant SAMD9134-385 or SAMD9156-385 protein was expressed in Escherichia coli as a SUMO fusion with a 6xHis tag and purified by nickel nitrilotriacetic acid (Ni-NTA) as previously described (29). The complex of SAMD9156-385 and a 22-nt dsDNA was crystalized in a solution containing 0.1 M Heps, pH 7.5, 0.2 M ammonium acetate (NH4Ac), 20 mM MgCl2, and 20% PEG3350. Diffraction data from a selenomethionine-substituted protein crystal were used to obtain an initial structure by the single-wavelength-anomalous-dispersion method, while data from a native protein crystal were used to obtain a higher-resolution structure by the molecular replacement method. The binding of the purified SAMD9 proteins with a 5'-FAM-labeled 22-nt dsDNA was studied by EMSA on a 0.8% native agarose gel. Affinities were obtained by nonlinear regression curve fitting of the fluorescence polarization data. Plasmids for transient expression of mCherry and SAMD9/9L fusions, psDNA6.2/mCherry-SAMD9/9L, were a generous gift of Jeffery M. Klco, St. Jude Research Hospital, Memphis, TN (27). Specific mutations of SAMD9/9L were introduced into the plasmids through recombinant PCR-based site-directed mutagenesis as described previously (30). The plasmids were transfected into HEK 293T cells for 24 to 36 h. For assessing antiviral activities, the transfected cells were infected with vK1 C7 / GPP (20, 28, 30) for 15 h. For assessing antiproliferative activities, the transfected cells were treated with 5-ethyl-2'-deoxyuridine (EdU) for 2 h before fixation and processed with Click-IT Plus Edu Alexa Fluor 488 Clickable O-nitrotyrosine Assay Kit (Thermo Fisher Scientific). For assessing protein synthesis level, transfected cells were treated with O-propargyl-puromycin (OPP) for 30 min before they were processed with Click-IT Plus OPP Alexa Fluor 488 Protein
Synthesis Assay Kit (Thermo Fisher Scientific). For assessing protein elongation rate, identically transfected cells were treated with 2 μg/mL Harringtonine (LKT Laboratories, Inc.) for various times before the cellular protein synthesis level was assessed with Click-IT Plus OPP kit. Flow cytometry analysis was performed with an LSR-II cell analyzer (BD Biosciences). For RNA-seq (RNA-seq) analysis, BT20 cells transduced with SAMD9-R1293W mutants exemplified significantly changed mRNAs were analyzed by PANTHER (32) to search enriched functional repression. identically transfected cells were treated with 2 μg/mL doxycycline for 24 h. Messenger RNA (mRNA) was isolated from the cells and processed for DNA library construction with NEBNext Ultra RNA Library Prep Kit for Illumina (NEB). RNA-seq reads were mapped to the human transcriptome and counted for each transcript. The count table was analyzed by DESeq2 (31), and the significantly changed mRNAs were analyzed by PANTHER (32) to search enriched functional interactions. The more detailed materials and methods can be found in SI Appendix, Materials and Methods.

Data Availability. Crystal structure data have been deposited in Protein Data Bank (Accession No. 7ksq). RNA-seq data have been deposited in Gene Expression Omnibus (GSE186456). All other study data are included in the article and/or supporting information.

ACKNOWLEDGMENTS. We thank the staff of beam-line 19ID at the Advanced Photon Source for their support and Dr. Jeffrey M. Klco for providing plasmids. This work was supported by NIH Grant No. AI151638 (Y.X.). J.D. is also supported by the Oklahoma Agricultural Experiment Station at Oklahoma State University under Project No. OKL00360. Flow cytometry data were generated in the University of Texas (UT) Health San Antonio Flow Cytometry Shared Resource Facility (supported by Grant Nos. NIH P30 CA054174 and U1L TR002645) with the help from Sebastian Montagnino. Sequencing data were generated in the UT Health San Antonio Genome Sequencing Facility (supported by Grant Nos. NIH P30 CA054174 and 15100D021805-01).

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