



Linking nuclear matrix–localized PIAS1 to chromatin SUMOylation *via* direct binding of histones H3 and H2A.Z

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As a conserved posttranslational modification, SUMOylation has been shown to play important roles in chromatin-related biological processes including transcription. However, how the SUMOylation machinery associates with chromatin is not clear. Here, we present evidence that multiple SUMOylation machinery components, including SUMO E1 proteins SAE1 and SAE2 and the PIAS (protein inhibitor of activated STAT) family SUMO E3 ligases, are primarily associated with the nuclear matrix rather than with chromatin. We show using nuclease digestion that all PIAS family proteins maintain nuclear matrix association in the absence of chromatin. Of importance, we identify multiple histones including H3 and H2A.Z as directly interacting with PIAS1 and demonstrate that this interaction requires the PIAS1 SAP (SAF-A/B, Acinus, and PIAS) domain. We demonstrate that PIAS1 promotes SUMOylation of histones H3 and H2B in both a SAP domain– and an E3 ligase activity–dependent manner. Furthermore, we show that PIAS1 binds to heat shock–induced genes and represses their expression and that this function also requires the SAP domain. Altogether, our study reveals for the first time the nuclear matrix as the compartment most enriched in SUMO E1 and PIAS family E3 ligases. Our finding that PIAS1 interacts directly with histone proteins also suggests a molecular mechanism as to how nuclear matrix–associated PIAS1 is able to regulate transcription and other chromatin-related processes.

SUMO (Small Ubiquitin-related MOdifier) modification (SUMOylation) is a conserved posttranslational modification uniquely present in eukaryotes. SUMOylation plays important roles in various biological processes including transcriptional regulation, cell cycle, genome stability, DNA damage repair, cell motility, and metabolism (1–6). SUMOylation is a multi-step process that involves an activating heterodimer E1 enzyme (SAE1/SAE2), a single conjugating E2 enzyme UBC9 and a limited number of E3 enzymes that promote transfer of activated SUMO from E2 to substrates (2, 7).

Global proteomic studies of SUMOylated proteins indicate that, although SUMOylated proteins can be identified in nearly all cellular compartments, SUMOylation is a predominantly nuclear event and occurs primarily in transcription factors, chromatin-associated proteins, and RNA-binding proteins (8–12). In addition, histones, the central components of chromatin, are subjected to SUMOylation both in yeast and mammalian cells and histone SUMOylation is typically associated with transcriptional repression (13–17). Extensive studies on transcription factor and cofactor SUMOylation have established SUMOylation as a major mechanism for transcriptional regulation, most likely due to the potential marked impact of SUMOylation on protein–protein interaction, conformation, and subcellular localization of substrate proteins (1, 5, 16, 18–20). Given the functional significance of SUMOylation in transcription, several studies have investigated the genome-wide distribution of SUMOylated proteins as well as SUMO-modification enzymes under both regular and stressed conditions. Collectively these studies have revealed that SUMOylated proteins, SUMO E2 enzyme UBC9, and some SUMO E3 proteins are highly enriched at promoters of actively transcribed genes and that SUMOylation serves to suppress the transcriptional scale of the actively transcribed genes (21–23). These observations are in agreement with the previous findings that SUMOylation is highly correlated with transcriptional repression (1, 24, 25). However, these studies also identified a substantial portion of chromatin that is enriched of SUMOylated proteins but apparently lacks SUMO enzymes, raising the possibility that SUMOylation within these chromatin regions is not catalyzed by local chromatin-associated SUMO enzymes.

The PIAS family proteins are the major SUMO E3 ligases (26, 27). Human PIAS family genes have four members, PIAS1, PIASx (PIAS2), PIAS3, and PIASy (PIAS4). PIAS proteins contain five common structural domains, namely, the SAP domain, PINIT sequence, RING domain, SIM sequence, and C-terminal S/T region. The SAP domain can bind the nuclear matrix attachment sequence rich in A-T bases (28), whereas the RING domain can interact with UBC9 and helps SUMO transfer from UBC9 to the lysine residue of substrates (29). PIAS1, the founder member of this family protein, was initially

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identified as a protein inhibitor of activated STAT1 (30). Since then, PIAS1 has been shown to interact with various transcription factors and coregulators including AR and HDACs and plays regulatory roles in transcription, DNA damage response, and other processes (23, 31–34). Like PIAS1, other PIAS proteins also have roles in transcriptional regulation, genome stability, and other processes. Interesting, a previous study reported that PIASy, also known as PIAS4, is a nuclear matrix protein (35, 36). However, it is unknown whether other PIAS proteins also associate with the nuclear matrix. Furthermore, this observation also raises an intriguing question as to how the PIAS family proteins contribute to chromatin SUMOylation if they are localized within the nuclear matrix.

In this study, we present evidence that all PIAS family E3 ligases are primarily associated with the nuclear matrix but not chromatin. In addition, the SUMO E1 proteins SAE1 and SAE2 are also highly enriched in the nuclear matrix. We focused on PIAS1 for the detailed study and provided multiple lines of evidence that PIAS1 resides primarily within the nuclear matrix. We identified PIAS1 direct interacting proteins through an approach involving short-arm chemical cross-linking followed by immunoaffinity purification and mass spectrometry analysis. This revealed core histones H3 and variant H2A.Z as the most abundant PIAS1-interacting proteins. Furthermore, we showed that PIAS1 promotes SUMOylation of histones H3 and H2B in a SAP domain- and Ring domain-dependent manner. Our finding that PIAS1 interacts directly with histones provides a novel mechanism as to how a nuclear matrix-localized PIAS protein could function in chromatin-related biological processes *via* promoting histone SUMOylation and possibly also chromatin-associated nonhistone SUMOylation.

Results

SUMO E1 subunits SAE1/SAE2 and E3 ligase PIAS1 localize primarily in the nuclear matrix

The finding that PIASy is associated with the nuclear matrix (35, 36) prompted us to examine whether other components of the SUMO machinery also localize to nuclear matrix. Currently there is no consensus protocol for preparation of the nuclear matrix fraction. To this end, we first employed a commonly used biochemical subcellular fractionation protocol to separate HeLa cellular components into cytosol, nuclear extract, and nuclear matrix plus chromatin (Fig. 1A) (37). We validated our fractionation protocol by Western blot analysis using antibodies against the cytosolic marker GAPDH, nuclear marker lamin A/C, and chromatin marker histone H3 (Fig. 1B). We then analyzed the distribution of two SUMO E1 subunits SAE1 and SAE2, E2 UBC9, and E3 PIAS1 in these three fractions by Western blotting. The representative results in Figure 1B showed that PIAS1 and SAE1/2 were detected primarily in the nuclear matrix plus chromatin fractions, whereas UBC9 could be detected in both nuclear extract and nuclear matrix plus chromatin fractions. To determine if the above SUMO enzymes were associated with chromatin and/or the nuclear matrix, we utilized a protocol as illustrated in

Figure 1C that removes chromatin from the nuclear matrix (38–40). In this protocol, soluble chromatin was released from nuclear pellets by DNase I digestion followed by 0.25 M ammonium sulfate washes, and the remaining chromatin was further extracted from the pellet by 2 M NaCl wash to generate the chromatin-free, high-salt-resistant nuclear matrix. The successful depletion of chromatin from the nuclear matrix could be evaluated by the lack of histone H3 in the remaining nuclear pellet and the presence of H3 in 2 M NaCl wash fraction, as shown in Figure 1D. Using this approach, we observed that PIAS1 was present primarily in the nuclear matrix rather than in the chromatin fractions with histone H3. Similarly, the majority of SAE1 and SAE2 was detected in the nuclear matrix. On the other hand, UBC9 was detected in the cytosol, nuclear extract plus soluble chromatin, and the 2 M NaCl wash but was not detected in the nuclear matrix (Fig. 1D). Although this experiment supports UBC9 as a chromatin-associated protein, it reveals surprisingly that PIAS1 and SAE1/SAE2 are primarily localized in the nuclear matrix.

To test further if PIAS1 and SAE1/SAE2 indeed exist primarily in the nuclear matrix, we used a modified cellular fractionation protocol that releases chromatin from nuclear pellets by Benzonase digestion (Fig. 1E). As shown in Figure 1F, Benzonase treatment released more than 60% of histone H3 and nearly all UBC9 from the pellet, whereas the majority of PIAS1 and SAE1/SAE2 remained in the pellet fraction, indicating that they were indeed associated primarily with the nuclear matrix. Although different protocols yielded inconsistent results on Lamin A/C subcellular localization, from the soluble nuclear fraction in protocol A to complete nuclear matrix association in protocol B, PIAS1 and SAE1/SAE2 were consistently detected predominantly in the nuclear matrix. Taken together, these cellular fractionation experiments reveal the nuclear matrix as the major cellular compartment where PIAS1 and SAE1/SAE2 reside.

To further confirm the above cellular fractionation-based observation, we examined the association of PIAS1, SAE1, and UBC9 with the nuclear matrix or chromatin by immunofluorescence staining. We treated HeLa cells with 0.5% Triton X-100 to permeabilize cells and then treated with Benzonase for 4 h, followed by washes to remove chromatin. The cells were then processed for immunofluorescence staining of PIAS1 and DAPI staining of DNA. As shown in Figure 1G, we found that the cells were essentially depleted of DNA upon 4-h Benzonase treatment, indicating that bulk chromatin was released and washed out of the cells. However, PIAS1 and SAE1 were detected mainly as bright nuclear foci in both control and Benzonase-treated cells, yet UBC9 was not detected after Benzonase treatment, demonstrating further that bulk nuclear PIAS1 and SAE1, but not UBC9, are nuclear matrix associated.

To examine if nuclear matrix localization is a common feature of PIAS1 and SAE1/SAE2, we also analyzed their subcellular localization in mouse primary hepatocytes using the fractionation protocol in Figure 1C. As shown in Figure 2A, PIAS1 and SAE1/SAE2 were mainly detected in the

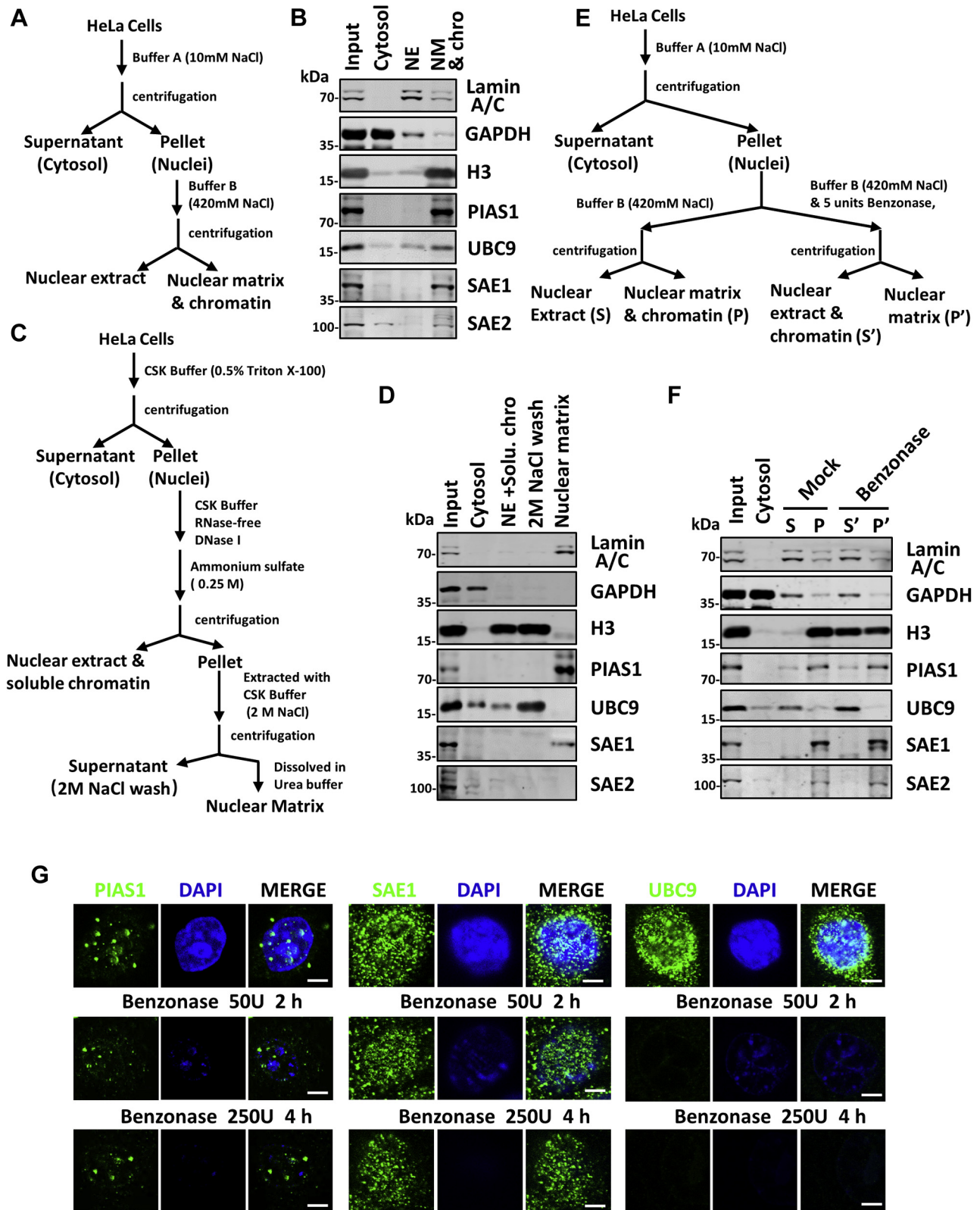


Figure 1. The SUMO E1 activating subunits SAE1/SAE2 and E3 ligases PIAS1 are mainly detected in the nuclear matrix. *A*, a common scheme for fractionation of cellular components into cytosol, nuclear extract, and nuclear matrix and chromatin. *B*, HeLa cells were subjected to cellular fractionation as illustrated in (*A*) and Western blot analyses were performed for cytosol marker GAPDH, nuclear and nuclear matrix marker Lamin A/C, chromatin marker histone H3, and SUMO machinery components SAE1/SAE2, UBC9, and PIAS1. *C*, experimental scheme for preparation of chromatin-free nuclear matrix. *D*, HeLa cells were fractionated as illustrated in (*C*), and the resulting fractions were analyzed by Western blotting using antibodies as indicated. *E*, experimental scheme with Benzonase treatment; fractions obtained were cytosol, nuclear extract (S), nuclear matrix and chromatin (P), nuclear extract plus soluble chromatin after Benzonase digestion (S'), nuclear matrix plus remaining insoluble chromatin after Benzonase digestion (P'). *F*, HeLa cells were fractionated as in (*E*) and analyzed by Western blotting using antibodies as indicated. *G*, representative immunofluorescence images of HeLa cells treated with or without Benzonase as indicated and stained with antibodies specific to PIAS1, SAE1, and UBC9, respectively. DAPI staining showed that DNA was essentially depleted after Benzonase treatment. The scale bar represents 5 μm. NE, nuclear extract; NM & chro, nuclear matrix and chromatin.

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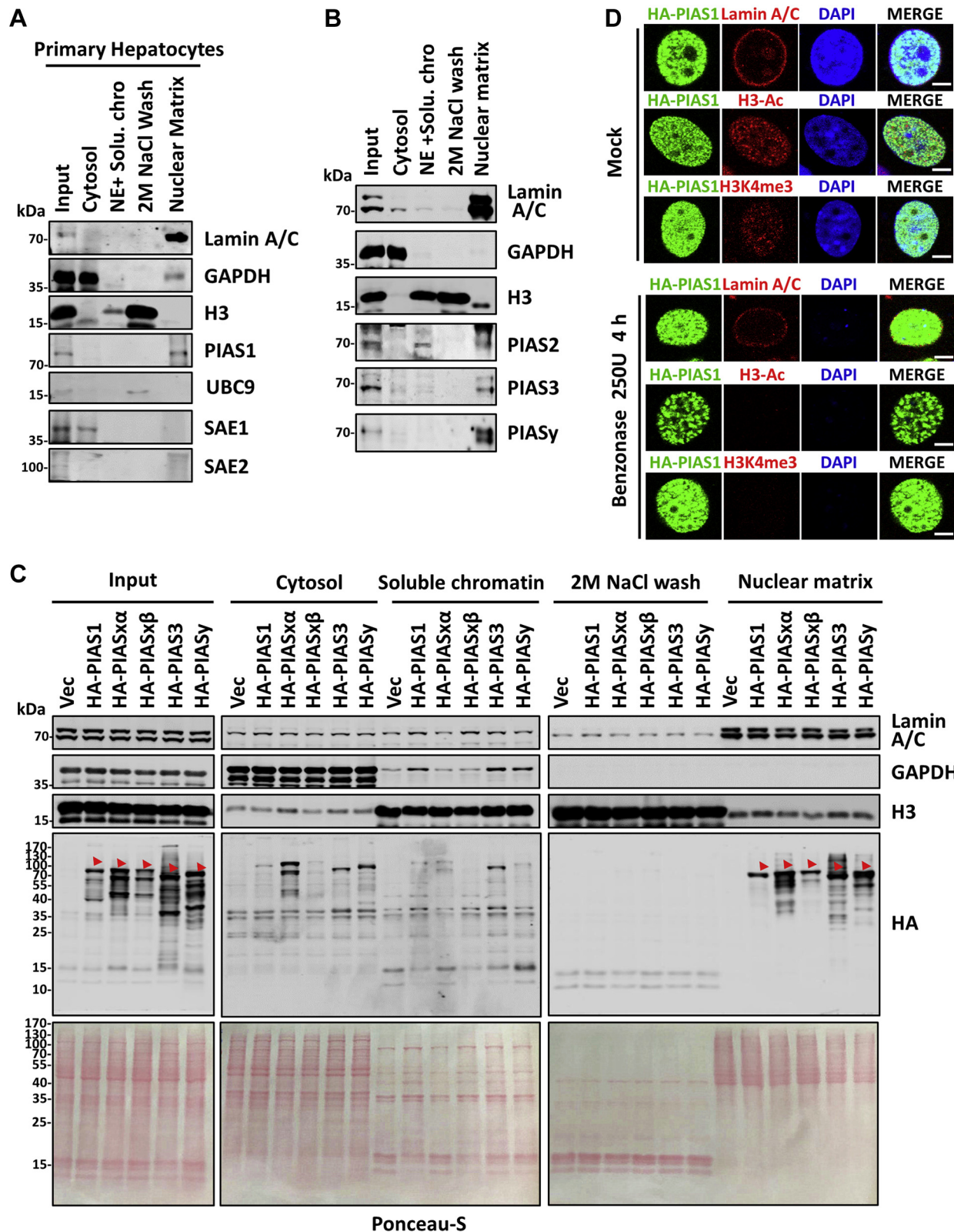


Figure 2. Nuclear matrix localization of ectopically expressed PIAS family E3 ligases. A, mouse primary hepatocytes were subjected to subcellular fractionation by DNase I digestion plus the high-salt extraction as illustrated in Figure 1C, and the distribution of SAE1/SAE2, UBC9, and PIAS1 was analyzed by Western blotting. B, HeLa cells were fractionated by DNase I digestion plus the high-salt extraction as illustrated in Figure 1C. Western blot analysis indicates that PIAS2, PIAS3, and PIASy proteins were mainly detected in the nuclear matrix fraction. C, HeLa cells were transfected with various plasmid encoding HA-tagged PIAS proteins as indicated. Two days after transfection the cells were collected and subjected to fractionation as illustrated in Figure 1C. The resulting fractions were analyzed by Western blotting using antibodies as indicated. Also shown are Ponceau-S staining images showing the relative levels and distinct patterns of proteins in different fractions, “◀” marks the position of full-length PIAS family E3 ligases. D, representative immunofluorescence images showing the levels and nuclear staining patterns of ectopically expressed HA-PIAS1 were not affected upon removal of

nuclear matrix of mouse primary hepatocytes as well. Taken together, these data indicate that PIAS1 and SAE1/SAE2 are mainly localized in the nuclear matrix, whereas UBC9 appears to be primarily associated with chromatin.

All PIAS family proteins mainly reside in the nuclear matrix

Next, we examined if nuclear matrix localization is a common feature for all PIAS SUMO E3 ligase family members. To this end, we first used the protocol in Figure 1C to prepare nuclear matrix fractions depleted of chromatin. Subsequent Western blot analysis showed that, like PIAS1 in Figure 1D, PIAS2, PIAS3, and PIASy in HeLa cells were all mainly detected in the nuclear matrix fraction (Fig. 2B). To consolidate this observation, we ectopically expressed each HA-tagged PIAS family member in HeLa cells and examined its subcellular localization following the same cellular fractionation protocol as illustrated in Figure 1C. Representative Western blot results in Figure 2C clearly showed that ectopically expressed PIAS1, PIASx α , PIASx β , PIAS3, and PIASy were all mainly resided in the nuclear matrix.

To test further the association of ectopically expressed PIAS proteins with the nuclear matrix, we also carried out immunofluorescence staining upon treating permeabilized cells without or with Benzonase to remove chromatin. Representative results in Figure 2D and Fig. S1 showed that Benzonase treatment resulted in nearly complete loss of the DAPI signal, acetylated H3, as well as trimethylated H3K4, demonstrating the effective removal of chromatin from Benzonase-treated cells. However, Benzonase treatment did not significantly affect the levels and patterns of nucleus-localized PIAS1 proteins (Fig. 2D) and all other PIAS proteins (Fig. S1), further supporting that all PIAS proteins are mainly associated with the nuclear matrix.

Taken together, our results suggest that all members of the PIAS family proteins are primarily nuclear matrix associated.

Identification of PIAS1 direct interacting proteins through DSP-based cross-linking

The above results provide evidence that the PIAS family proteins and SUMO E1 enzymes mainly exist in the nuclear matrix compartment, raising a series of intriguing questions including how nuclear matrix-associated SUMO enzymes regulate chromatin-related functions (1, 5, 41). To begin to address this issue, we attempted to identify the direct interacting proteins of PIAS1, a major SUMO E3 ligase. We made use of the cross-linking reagent dithiobis succinimidyl propionate (DSP), which is a water-insoluble, membrane-permeable homobifunctional N-hydroxysuccinimide ester that can reversibly cross-link different proteins by primary amines in cells (42, 43). As DSP is a short-arm cross-linker, the proteins cross-linked by DSP are generally considered as direct interacting proteins. To identify PIAS1-interacting

proteins by DSP-based cross-linking, we employed a protocol illustrated in Figure 3A. We ectopically expressed HA-PIAS1 in HeLa cells and treated live HeLa cells without or with DSP. As PIAS1 localizes primarily in the nuclear matrix, nuclei were prepared and solubilized under a denatured condition in buffer containing 1% Triton X-100 and 7 M urea. After centrifugation, HA-PIAS1 and its covalently cross-linked interacting proteins were immunoaffinity purified from the soluble fraction with anti-HA antibody beads. The resulting proteins were visualized by silver staining (Fig. 3B, left panel) and Western blot analysis revealed that a similar level of HA-PIAS1 was obtained under both cross-linking and no cross-linking conditions (Fig. 3B, right panel). Both samples were subjected to protein identification by mass spectrometry analysis. A total of 129 proteins were identified in the sample without DSP cross-linking, whereas 240 proteins were identified in the sample with DSP cross-linking, with 85 proteins overlapped between the two groups. We considered proteins identified to a similar extent in the sample without DSP cross-linking as nonspecific, and we identified 155 potential PIAS1 direct interacting proteins after removing these overlapped proteins (Fig. 3C and Table S1). To our delight, on the top list of potential PIAS1 direct interacting proteins are histone proteins H3, H2A.Z, H2B, H2A, H1, and macro-H2A (Fig. 3D). Considering that the histone variant H2A.Z accounts for less than 10% of total H2A, the result that a similar number of H2A.Z and H2A peptides were identified within the PIAS1-associated proteins implies a preferential interaction between PIAS1 and H2A.Z. GO analysis revealed that the identified PIAS1 direct interacting proteins are highly enriched in RNA binding, protein-protein interaction, and nucleosomal DNA binding (Fig. S2), consistent with the broad roles of SUMOylation in transcription, RNA processing, and regulation of protein-protein interaction.

Validation of the interaction between PIAS1 and H3 and H2A.Z

The identification of multiple histone proteins as potential PIAS1 direct interacting proteins suggests that nuclear matrix-associated PIAS1 may regulate chromatin function by direct binding to histones. H2A.Z is a replication-independent histone variant and is involved in regulation of transcription, DNA repair, and heterochromatin function (44, 45). As H3 and H2A.Z are the top two among the potential PIAS1 direct interacting histone proteins, we next focused on validating if PIAS1 interacts directly with H3 and H2A.Z. We ectopically coexpressed FLAG-H3 or FLAG-H2A.Z with HA-PIAS1 in HEK293T cells, and 24 h after transfection the cells were treated with or without DSP. The cells were lysed and sonicated under a denaturing condition to solubilize matrix-associated PIAS1 proteins and histones from chromatin. The resulting soluble extracts were prepared and used for

chromatin by Benzonase treatment. Note that Benzonase treatment resulted in depletion of not only DAPI signal but also acetylated H3 and H3K4me3 signals, confirming a near-complete depletion of chromatin. Also note that Benzonase treatment did not affect the level as well as perinuclear pattern of Lamin A/C. The scale bar represents 5 μ m.

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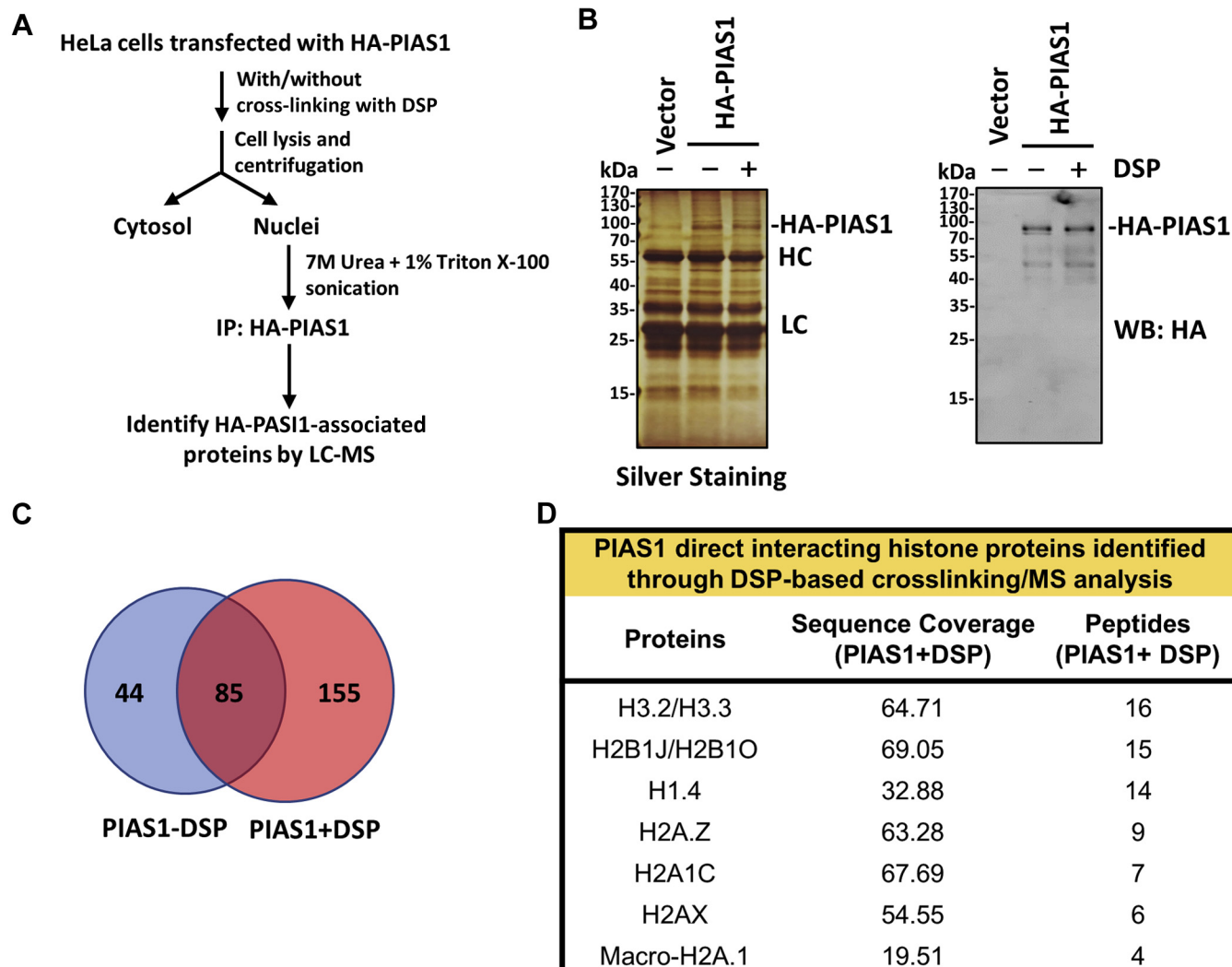


Figure 3. Identification of PIAS1 direct interacting proteins through DSP-based cross-linking. A, schematic diagram of experimental approach for purification and identification of PIAS1 direct interacting proteins. B, silver staining gel image of proteins copurified with HA-PIAS1 under condition with or without DSP cross-linking (left). The positions of antibody heavy chains (HC) and light chains (LC) are indicated. Also indicated is the position of HA-PIAS1. The PIAS1-interacting proteins were not obvious in the silver staining gel owing to the presence of a large amount of antibodies. C, Venn diagram showing the number of proteins identified together with immunoprecipitated HA-PIAS1 under conditions with or without DSP cross-linking. As purification of HA-PIAS1 was carried out under denaturing condition, the copurified proteins identified without DSP cross-linking were considered as nonspecific and were removed from the list of proteins identified with DSP cross-linking. D, the list of top HA-PIAS1 direct interacting histones and nonhistone proteins. The proteins were shown according to their relative numbers of identified peptides.

coimmunoprecipitation (co-IP) assay analyzing the interaction between HA-PIAS1 and FLAG-H3 or FLAG-H2A.Z. As shown in Figure 4A, FLAG-H3 was coimmunoprecipitated with HA-PIAS1 only when cells were treated with DSP. Similarly, representative results in Figure 4B showed that FLAG-H2A.Z was coimmunoprecipitated with HA-PIAS1 only in the sample treated with DSP. These co-IP experiments therefore validated the interaction between PIAS1 and histones H3 and H2A.Z. The lack of co-IP between HA-PIAS1 and FLAG-H3/FLAG-H2A.Z in the absence of DSP treatment indicated that their protein-protein interactions were abolished under the denaturing condition used for cell extract preparation. Denaturation is necessary because association with the nuclear matrix precluded us from examining the interaction between PIAS1 and histones using soluble cell extracts under nondenaturing conditions.

To test if endogenous PIAS family proteins bind histones H3 and H2A.Z, HeLa cells were treated with DSP and processed for immunoprecipitation-Western blot analysis under the denaturing condition as above. Subsequent co-IP experiments demonstrated that endogenous PIAS1 is associated with both histones H3 and H2A.Z. We could not unambiguously detect PIAS2 in this assay, as the endogenous PIAS2 level was low. On the other hand, we did not observe significant co-IP of PIAS3 with either H3 or H2A.Z, whereas PIASy might weakly interact with H2A.Z (Fig. 4C).

To test further the interaction between PIAS1 and histones, we performed GST pulldown assay using GST-PIAS1 fusion protein and cellular extracts derived from FLAG-H3- or FLAG-H2A.Z-expressing HEK293T cells prepared as described above but under nondenaturing condition. Representative results in Figure 4, D and E showed that GST-PIAS1,

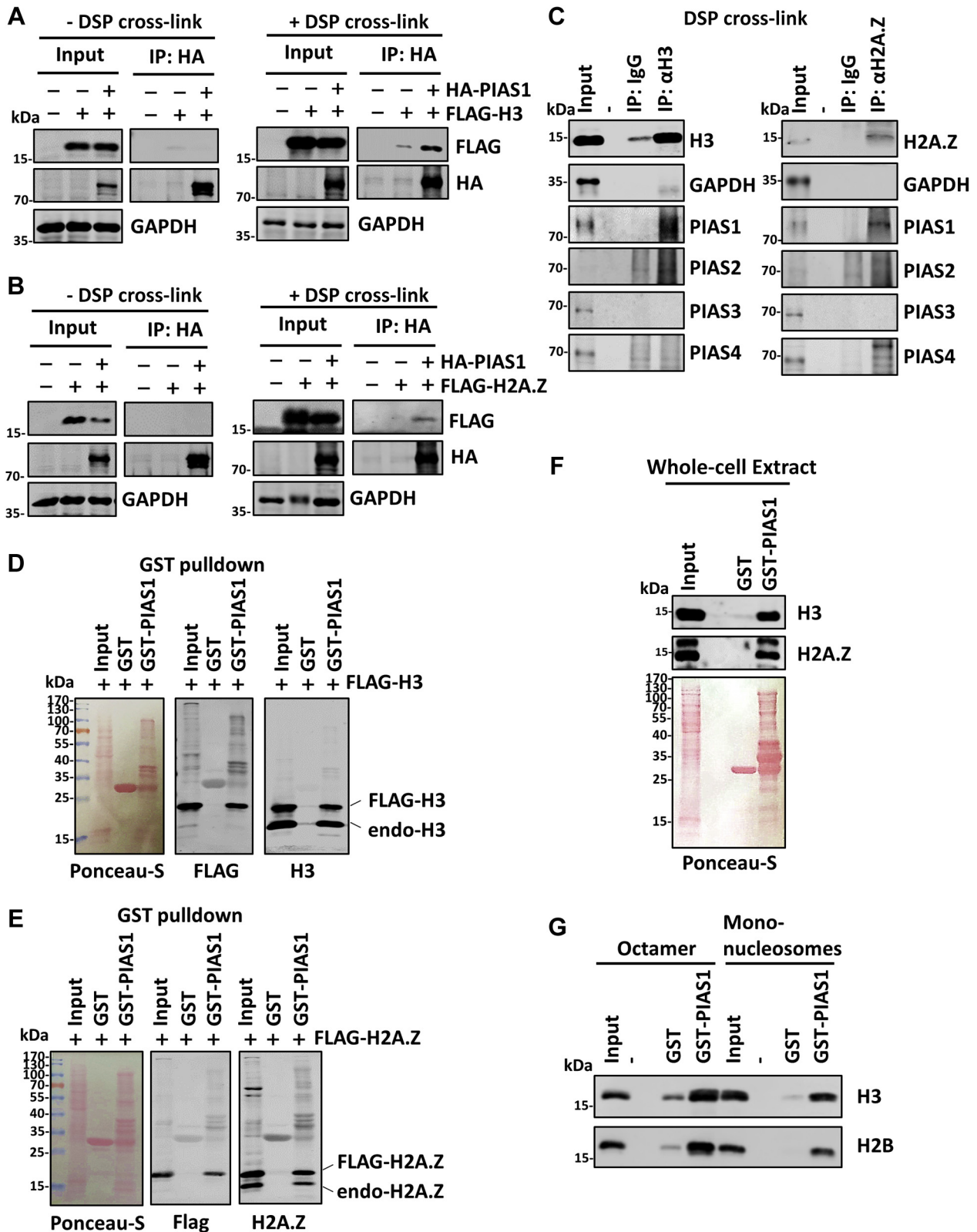


Figure 4. Validation of the direct interaction between PIAS1 and histones H3 and H2A.Z. *A*, immunoprecipitation (IP)-Western blot analysis confirming an interaction between ectopically expressed PIAS1 and histone H3. FLAG-H3 was expressed alone or together with HA-PIAS1, and the cells were treated without (*left*) or with (*right*) DSP, followed by IP under denaturing condition and Western blot analysis using antibodies as indicated. Note FLAG-H3 was coimmunoprecipitated with HA-PIAS1 only upon DSP treatment. *B*, IP-Western blot analysis confirming an interaction between ectopically expressed PIAS1 and histone H2A.Z. The experiments were as in (*A*) except FLAG-H2A.Z was used. *C*, IP-Western blot analysis showing the interaction between endogenous PIAS family proteins and histones H3 (*left panel*) and H2A.Z (*right panel*). HEK293T cells were treated with DSP and IP-Western blot analyses were carried out using antibodies as indicated. *D*, *in vitro* GST pull-down assays showing that PIAS1 binds both ectopically expressed FLAG-H3 as well as endogenous H3. HEK293T cells transfected with FLAG-H3 were lysed and sonicated under nondenaturing condition to solubilize chromatin. After centrifugation, the

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but not control GST, bound effectively both FLAG-H3 and FLAG-H2A.Z as well as endogenous H3 and H2A.Z. The ability for PIAS1 to bind H3 and H2A.Z was independent of ectopic expression of H3 or H2A.Z, as GST-PIAS1 also effectively pulled down H3 and H2A.Z from cell extracts derived from untransfected HEK293T cells (Fig. 4F).

To test if PIAS1 directly binds histones in chromatin, we carried out GST pull-down experiments with *in vitro*-reconstituted histone octamers and mononucleosomes. Representative results in Figure 4G showed that histone octamers bound avidly to GST-PIAS1 but weakly to control GST. Of importance, recombinant mononucleosomes also bound robustly to GST-PIAS1 but poorly to control GST. These side-by-side experiments clearly demonstrate that PIAS1 is capable of directly binding core histones as well as nucleosomes *in vitro*. Altogether our results demonstrate that PIAS1 is able to bind histones and chromatin both *in vivo* and *in vitro*.

The SAP domain is required for binding H3 and H2A.Z by PIAS1 in chromatin

Next, we attempted to define the functional domain(s) required for binding histones H3 and H2A.Z by PIAS1. To this end, we constructed a set of GST-PIAS1 fusion proteins with truncation of various lengths of the PIAS1 C-terminal region (Fig. 5A). We then used GST pull-down assay to examine their interaction with histones H3 and H2A.Z. The results in Figure 5B showed that deletion of the PINIT sequence, RING domain, SIM sequence, and C-terminal S/T region did not significantly affect the binding of endogenous histones H3 and H2A.Z by PIAS1. Furthermore, GST pull-down assay showed that deletion of these domains also did not affect the binding of ectopically expressed FLAG-H3 and FLAG-H2A.Z (Fig. S3A). However, deletion of the SAP domain markedly reduced the ability of PIAS1 to bind H3 and H2A.Z (Fig. 5C).

To test whether the SAP domain is also required for binding of endogenous histones H3 and H2A.Z in chromatin in cells, we expressed HA-PIAS1 or HA-PIAS1 Δ SAP in HEK293T cells and carried out DSP cross-link-based co-IP experiments under a denaturing condition. We found that deletion of the SAP domain severely impaired the interaction between PIAS1 and histones H3/H2A.Z (Fig. 5D). We validated this result further by examining the interaction between ectopically expressed FLAG-H3 or FLAG-H2A.Z and HA-PIAS1 or HA-PIAS1 Δ SAP (Fig. S3, B and C). As cells have very low levels of free histones, the majority of ectopically expressed FLAG-H3 and FLAG-H2A.Z were expected to be incorporated into chromatin. Thus, these co-IP assays demonstrate that the SAP domain is required for PIAS1 binding to H3 and H2A.Z in cellular chromatin.

Finally, we tested if the SAP domain is required for direct binding of histone H3 *in vitro*. We found all GST-PIAS1

deletion mutants including GST-PIAS1 Δ SAP bound recombinant histone octamers to a similar extent in *in vitro* pull-down (Fig. 5E). However, binding of recombinant mononucleosomes was severely impaired for GST-PIAS1 Δ SAP in comparison with GST-PIAS1 and other deletion mutants (Fig. 5E). Taken together, we conclude that the SAP domain is required for PIAS1 to effectively bind chromatin in cells and nucleosomes *in vitro*.

PIAS1 enhances SUMOylation of histones H3, H2A, and H2B

Mammalian histone SUMOylation was first reported upon ectopic expression of SUMO proteins (13). In that study, SUMOylation was observed in H4, although weak SUMOylation of H2A, H2B, and H3 was also reported. However, despite extensive studies on SUMOylation, little is actually known about histone SUMOylation in mammalian cells, presumably owing to its low abundance and difficulty in detection. Our finding that PIAS1 binds directly to histones H3 and H2A.Z and possibly other histones prompted us to test if PIAS1 plays a role in histone SUMOylation. To this end, we first co-expressed HA-PIAS1 and GFP-SUMO1 together with different FLAG-tagged histone proteins in HEK293T cells and examined SUMOylation on histones by Western blot analysis using both anti-FLAG and histone-specific antibodies. Representative results showed that PIAS1 markedly enhanced SUMOylation on ectopically expressed FLAG-H3 (Fig. 6A) and FLAG-H2B (Fig. 6C). SUMOylation on FLAG-H2A was clearly detected by using anti-FLAG antibody, but it was not detected by using anti-H2A antibody, possibly owing to low reactivity of the H2A antibody used (Fig. 6B). However, PIAS1 failed to promote SUMOylation on H4 (Fig. 6D) and H2A.Z (Fig. 6E). Thus, despite its robust interaction with H2A.Z, PIAS1 is not able to enhance H2A.Z SUMOylation.

We next examined if binding of chromatin is required for PIAS1 to promote histone SUMOylation. We tested this with histone H3. As shown in Figure 6F, unlike the wildtype PIAS1, PIAS1 Δ SAP mutant not only failed to promote H3 SUMOylation but actually behaved as a dominant negative mutant to inhibit H3 SUMOylation. Furthermore, we found that the SUMO E3 ligase activity is also required for PIAS1 to promote H3 SUMOylation, as an E3 ligase-deficient PIAS1 mutant (PIAS1m) was unable to promote H3 SUMOylation (Fig. 6G).

To test if PIAS1 promotes SUMOylation on endogenous histones and whether this is dependent on the PIAS1 SAP domain, we transiently transfected His-tagged SUMO1 together with HA-PIAS1 or HA-PIAS1 Δ SAP into HEK293T cells. His-SUMO1-conjugated proteins were then isolated from cellular extracts by using Ni²⁺-NTA resin under the denatured condition, and SUMOylation on the endogenous histones was detected by Western blot analysis. This experiment revealed that ectopic expression of HA-PIAS1, but not

supernatants were subjected to GST pull-down assay as indicated. Also shown is Ponceau-S staining image of the blot used for Western blot analysis. E, *in vitro* GST pull-down assays showing that PIAS1 binds both ectopically expressed FLAG-H2A.Z as well as endogenous H2A.Z. The experiments were essentially as (D) except the cells expressing FLAG-H2A.Z were used. F, *in vitro* GST pull-down assays showing that PIAS1 binds endogenous H3 and H2A.Z. HEK293T cells were lysed and sonicated to solubilize chromatin. After centrifugation, the supernatants were subjected to GST pull-down assay as indicated. Also shown is Ponceau-S staining image of the blot used for Western blot analysis. G, *in vitro* GST pull-down assays showing that PIAS1 binds both recombinant histone octamer and mononucleosomes.

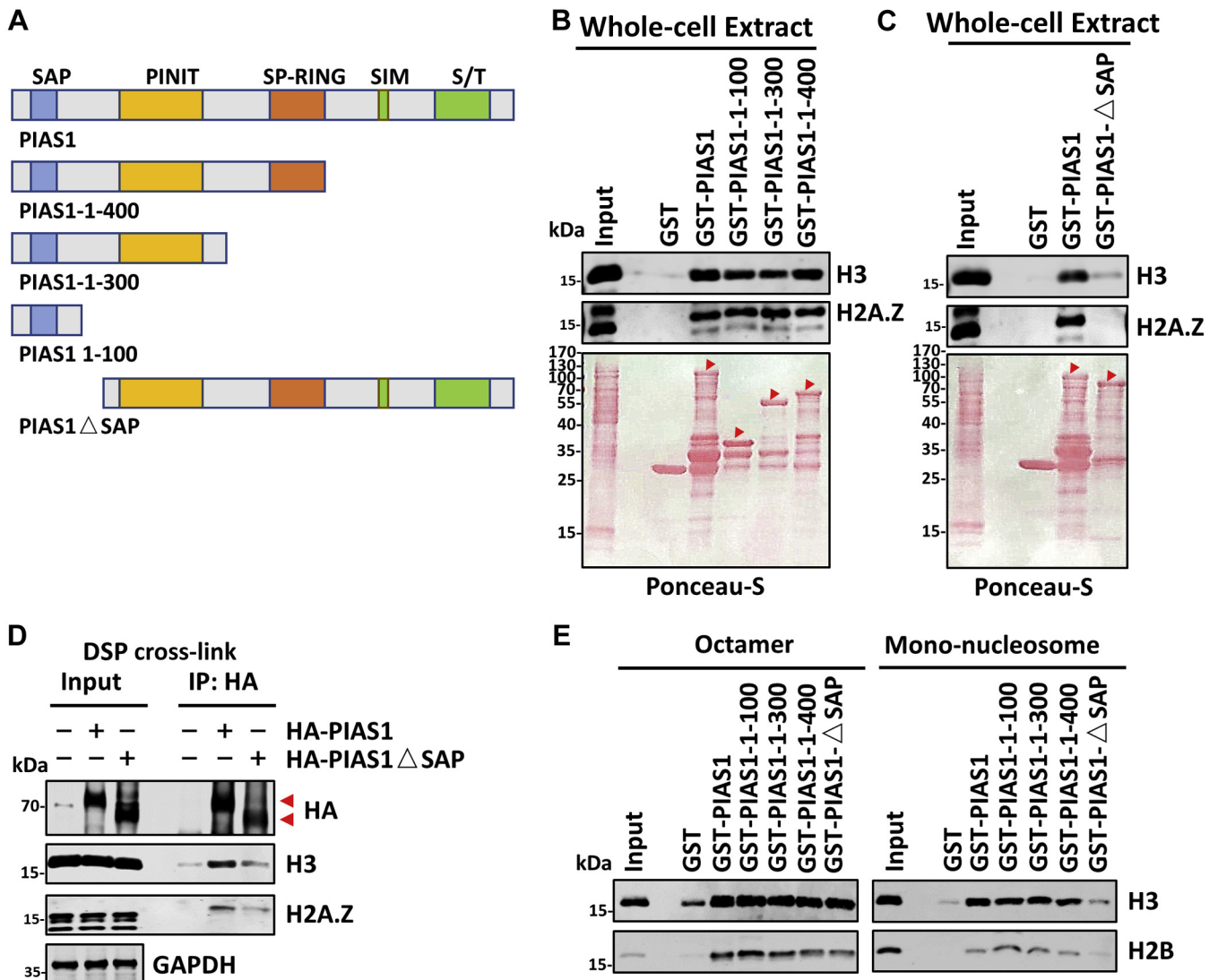


Figure 5. PIAS1 binds to H3 and H2A.Z mainly through its SAP domain. *A*, diagrams illustrating PIAS1 structural and functional domains and different truncated mutants. *B*, *in vitro* GST pull-down assay showing the binding of endogenous histones H3 and H2A.Z to GST-PIAS1 and different truncated mutants. Sonicated cell lysates prepared from HEK293T cells were subjected to *in vitro* pull-down assays using GST-PIAS1 or mutants as indicated. The bound proteins were subjected to Western blot analysis with anti-H3 or anti-H2A.Z antibody, respectively. Also shown is a Ponceau-S staining blot of purified GST or GST fusion proteins used for pull-down assay. “◀” marks the position of full-length PIAS1 and different truncated mutants. *C*, *in vitro* GST pull-down assays showing the binding of endogenous histones H3 and H2A.Z to PIAS1 and PIAS1 Δ SAP mutant. The experiments were carried out as in (B). “◀” marks the position of PIAS1 and PIAS1 Δ SAP mutant. *D*, IP-Western blot analysis showing deletion of SAP domain substantially impairs the interaction between PIAS1 and histones H3 and H2A.Z. HA-PIAS1 or HA-PIAS1 Δ SAP was expressed in HEK293T cells, and the cells were treated with DSP, followed by immunoprecipitation under denaturing condition with anti-FLAG antibody and Western blot analysis using antibodies as indicated. *E*, *in vitro* pull-down showing the effect of various deletions on binding of recombinant histone octamer and mononucleosomes. Note deletion of SAP has no obvious effect on PIAS1 binding of histone octamers, but impairs PIAS1 binding of mononucleosomes.

HA-PIAS1 Δ SAP, significantly enhanced SUMOylation on endogenous histones H3 and H2B (Fig. 7A, left panel), consistent with our results with ectopically expressed FLAG-H3 and FLAG-H2B. However, PIAS1 failed to promote SUMOylation on endogenous H2A, H4, and H2A.Z under the same condition (Fig. 7A, right panel). Using the same approach, we found that ectopic expression of HA-PIAS1 but not HA-PIAS1m enhanced histone SUMOylation on histones H3 and H2B (Fig. 7B). Together these experiments demonstrate that PIAS1 promotes SUMOylation on endogenous histones H3 and H2B in a SAP domain- and E3 ligase activity-dependent manner.

SAP domain is required for PIAS1 chromatin association and repression of heat shock-induced transcription in cells

Previous studies have shown that, under a heat shock condition, SUMOylated proteins rapidly accumulate at the promoters of HS-regulated genes and restrict the transcriptional activity of the HS-induced genes (21). Our finding that PIAS1 binds directly to histones H3 and H2A.Z and associates with chromatin in cells and nucleosomes *in vitro* in a SAP domain-dependent manner promoted us to test if our finding is functionally relevant in the context of HS-induced gene expression. To this end, we re-expressed HA-tagged wildtype PIAS1 or the PIAS1 mutant with deletion of the SAP domain

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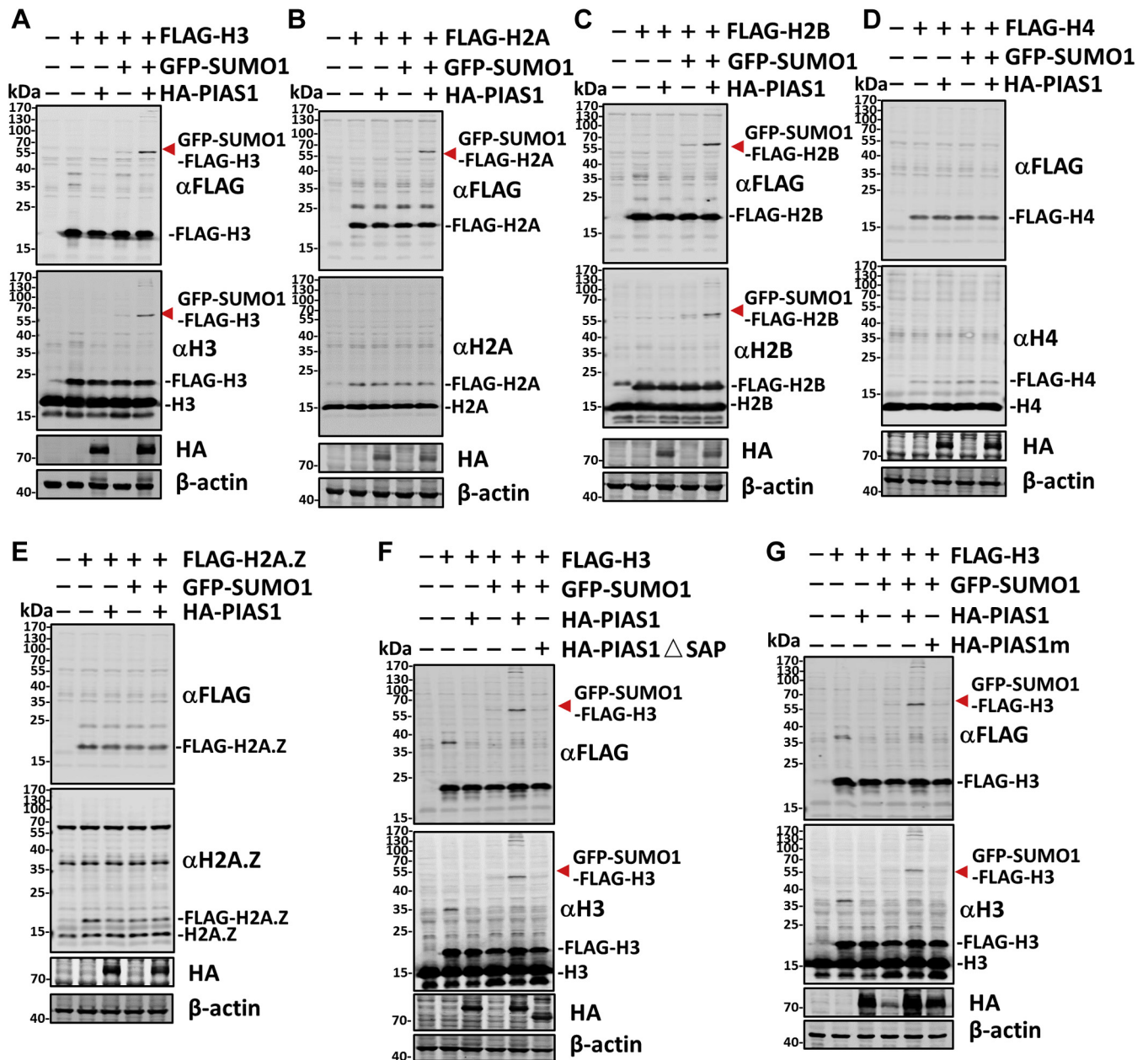


Figure 6. PIAS1 catalyzes SUMOylation on ectopically expressed histones H3, H2A, and H2B. A–E, individual FLAG-tagged histone constructs were coexpressed without or with GFP-SUMO1 and/or HA-PIAS1 in HEK293T cells, and SUMOylation on ectopically expressed FLAG-tagged and potential endogenous histones were analyzed by Western blotting using anti-FLAG and histone antibodies, respectively. Note that SUMOylation was detected on H3 by both anti-FLAG and anti-H3 antibodies (A), on H2A by anti-FLAG but not anti-H2A antibody (B), on H2B by both anti-FLAG and anti-H2B antibodies (C), not on H4 by both anti-FLAG and anti-H4 antibodies (D), and not on H2A.Z by both anti-FLAG and anti-H2A.Z antibodies (E). F, Western blot analysis showing that SAP domain is required for PIAS1 to catalyze histone H3 SUMOylation. FLAG-H3 was coexpressed without or with GFP-SUMO1, HA-PIAS1, or HA-PIAS1ΔSAP in HEK293T cells, and SUMOylation on H3 was detected by Western blot analysis using both anti-FLAG and anti-H3 antibodies. G, Western blot analysis showing that the E3 ligase activity is required for PIAS1 to catalyze histone H3 SUMOylation. The experiments were as in (F) except HA-PIAS1m was used.

in HeLa cells with PIAS1 knockout and subjected cells to HS treatment (Fig. 8A). Western blot analysis confirmed successful re-expression of HA-PIAS1 and HA-PIAS1ΔSAP with HA antibody as well as an antibody that was specific to either the PIAS1 N-terminal (PIAS1-N) or C-terminal region (PIAS1-C). Furthermore, Western blot analysis confirmed the effectiveness of HS treatment, as all HSF1 proteins were displayed as slower migrating, phosphorylated forms (Fig. 8A). Consistent with previous studies, RT-qPCR analysis

demonstrated that HS treatment induced substantial elevation of DNAJA1, HSPA6, and HSPA1A and HSPA1B transcripts and that expression of these genes was further augmented with PIAS1 knockout (Fig. 8, B–E). Of importance, we found that re-expression of HA-PIAS1 but not HA-PIAS1ΔSAP was able to partially suppress HS-induced transcriptional activation of these genes (Fig. 8, B–E). Chromatin immunoprecipitation (ChIP) assay using anti-HA antibody detected binding of PIAS1 at the promoters of these genes under the non-HS

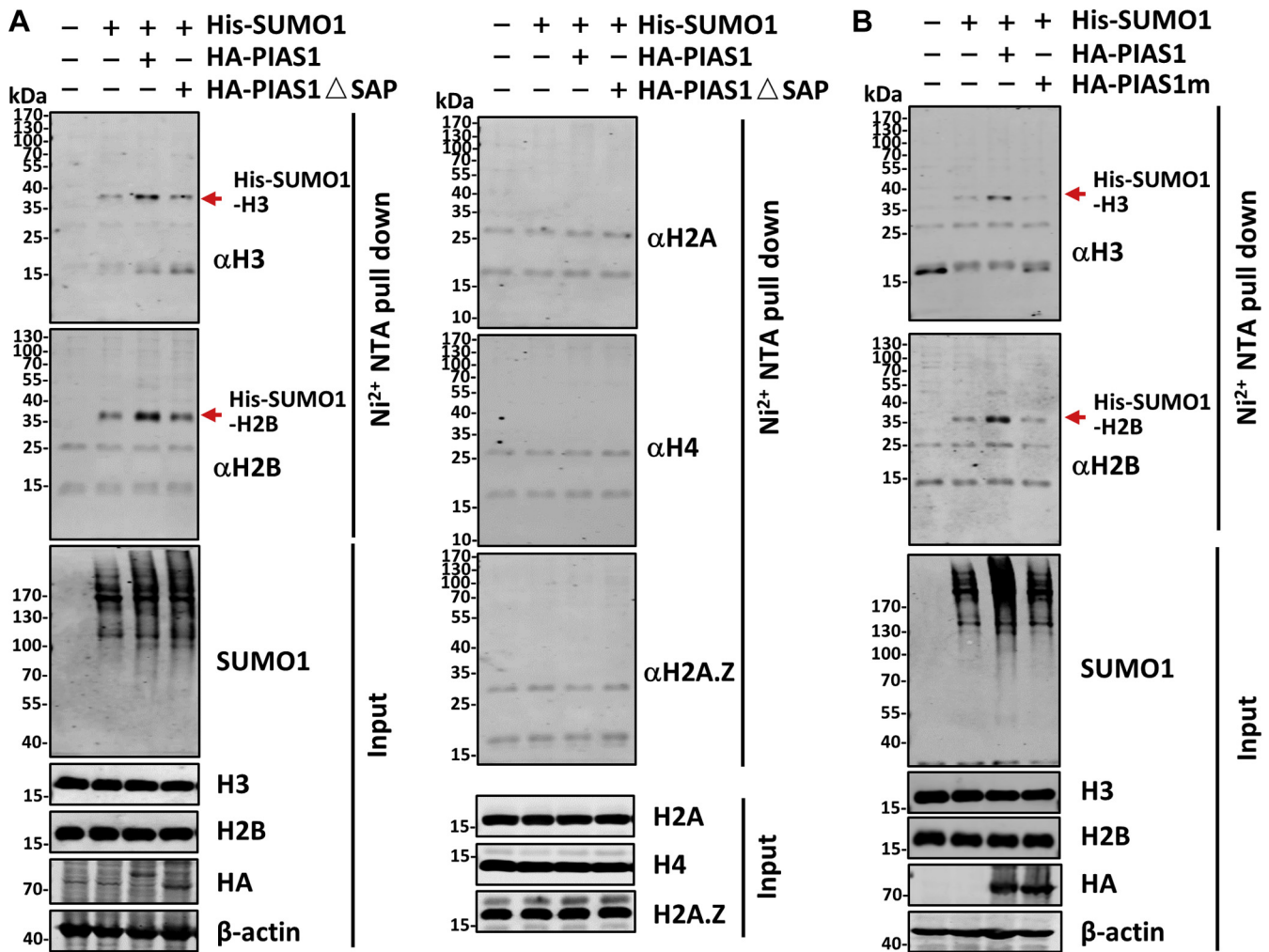


Figure 7. PIAS1 catalyzes SUMOylation on endogenous histones H3 and H2B. A, denaturing pull-down followed by Western blot analysis showing that PIAS1 promoted SUMOylation on endogenous histones H3 and H2B in a SAP domain-dependent manner. HEK293T cells were transfected with His-SUMO1 and HA-PIAS1 or HA-PIAS1ΔSAP as indicated. The proteins modified by His-SUMO1 were purified under denatured condition using Ni²⁺-NTA beads, and SUMOylation on histones was detected by Western blot analysis using anti-histone antibodies. B, denaturing pull-down followed by Western blot analysis showing that PIAS1 promoted SUMOylation on endogenous histones H3 and H2B in a E3 activity-dependent manner. The experiments were as in (A) except HA-PIAS1m was used.

condition. HS treatment resulted in a marked enhancement of PIAS1 binding at all four gene promoters (Fig. 8, F–I), in agreement with the previous result (21). However, no enrichment of HA-PIAS1ΔSAP was observed at these promoters under both non-HS and HS conditions (Fig. 8, F–I), despite a higher expression level of HA-PIAS1ΔSAP than HA-PIAS1 in this experiment (Fig. 8A). Together, qRT-PCR and ChIP assays demonstrated a critical role of the PIAS1 SAP domain in chromatin association and repression of transcription, especially under the HS condition.

Discussion

In an effort to characterize to what extent the SUMOylation machinery associates with chromatin, we demonstrate that the majority of SUMO E1-activating enzyme SAE1/SAE2 and PIAS family E3 ligases actually reside in the nuclear matrix rather than associate with chromatin (Figs. 1 and 2). However, we do find that the majority of UBC9 is associated with chromatin

(Fig. 1, D and F). Identification of PIAS1 direct interacting proteins reveals direct interactions between PIAS1 and multiple histone proteins (Fig. 3). This finding provides molecular explanation as to how nuclear matrix-associated PIAS proteins can promote SUMOylation of chromatin-associated histones and likely nonhistone proteins as well and consequently regulate chromatin-related biological processes.

The nuclear matrix is a nuclear skeletal structure that is believed to be involved in many nuclear functions including transcriptional regulation, DNA replication, and DNA damage repair (46), although the exact nature and composition of the nuclear matrix remain elusive (47). Practically, the nuclear ribonucleoproteinaceous structure that persists after DNA nuclease digestion and 2 M NaCl extraction to remove soluble nuclear proteins and chromatin is considered as the nuclear matrix (46). Using these criteria, we observe that the SUMO E1 subunits SAE1 and SAE2 and SUMO E3 PIAS family proteins, including PIAS1, mainly reside in the nuclear matrix in HeLa cells as well as in mouse primary hepatocytes (Figs. 1

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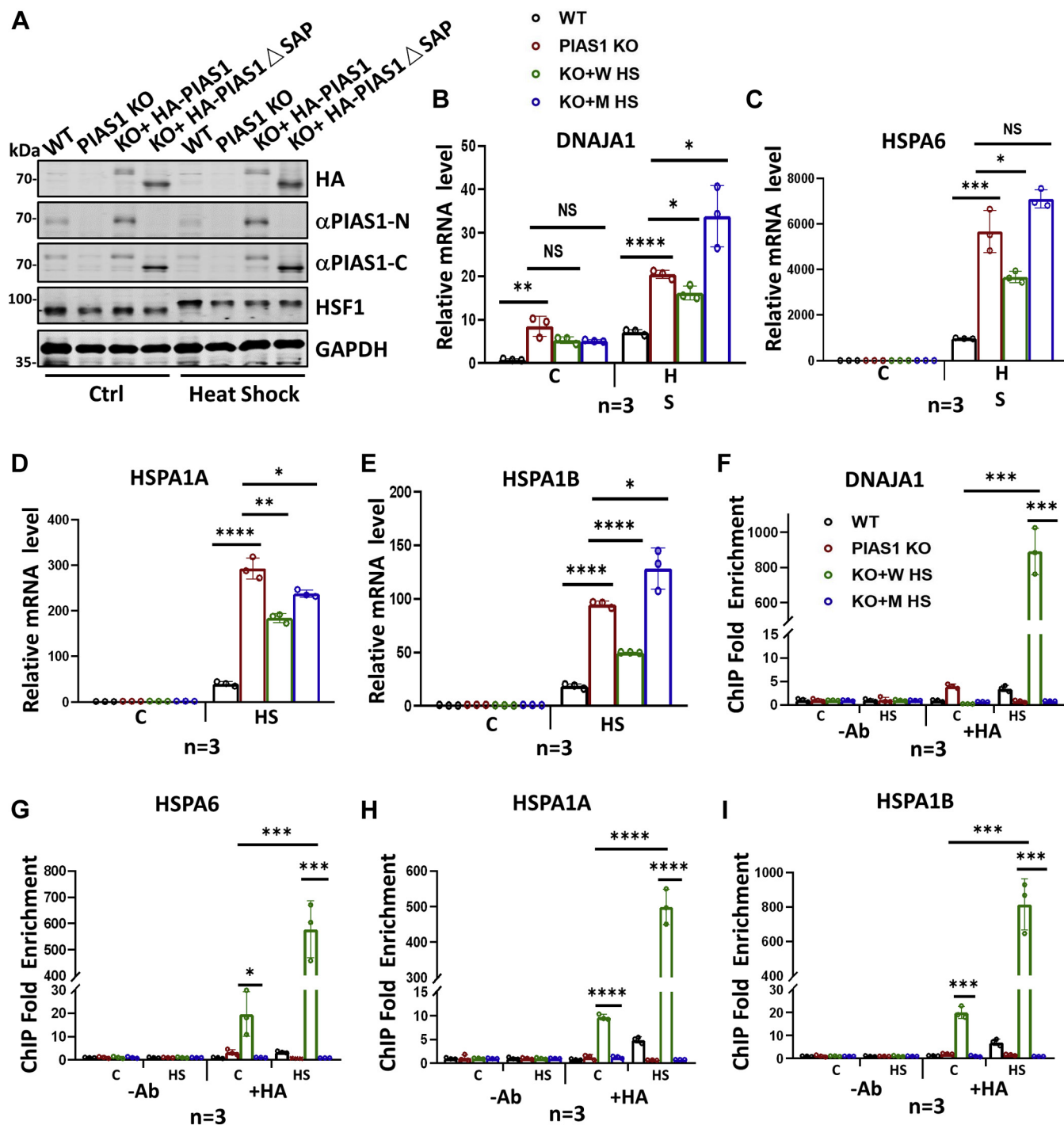


Figure 8. SAP domain is required for PIAS1 to suppress HS-induced transcription and association with chromatin. A, PIAS1-KO HeLa cells were re-expressed of HA-PIAS1 or HA-PIAS1 Δ SAP mutant as indicated, and expression of PIAS1 or PIAS1 Δ SAP was confirmed by Western blot analysis. HSF1 migrated more slowly on SDS-PAGE upon HS treatment; this was due to HS-induced hyperphosphorylation. B–E, RT-qPCR analysis showing the effect of re-expression of HA-PIAS1 or HA-PIAS1 Δ SAP on HS-induced transcriptional activation of DNAJA1 (B), HSPA6 (C), HSPA1A (D), and HSPA1B (E) in PIAS1-KO cells grown at control conditions (C; 37 °C) or exposed to heat shock (HS; 3 h at 42 °C). F–I, chromatin immunoprecipitation assay evaluating the promoter occupancy of HA-PIAS1 and HA-PIAS1 Δ SAP on the promoters of DNAJA1 (F), HSPA6 (G), HSPA1A (H), and HSPA1B (I) in PIAS1-KO cells grown at control conditions (C; 37 °C) or exposed to heat shock (HS; 40 min at 42 °C).

and 2). We explore this observation in detail with PIAS1 and demonstrate by both Western blot analysis and immunofluorescent staining that both endogenous and ectopically expressed PIAS1 proteins indeed reside mainly in the nuclear matrix (Figs. 1 and 2). Furthermore, all members of the PIAS

family proteins appear to mainly localize in the nuclear matrix, as shown with both endogenous and ectopically expressed PIAS family proteins (Fig. 2B and Fig. S1). Thus, nuclear matrix association is apparently not unique to PIASy as reported previously (36) but likely a common feature of PIAS

family SUMO E3 ligases. This finding, together with our observation that SAE1/SAE2 are also mainly localized in the nuclear matrix (Figs. 1 and 2A), raises an intriguing question as to whether SUMOylation plays a role in nuclear matrix formation and function. In this regard, SUMOylation is known to enrich at the PML body, which is associated with the nuclear matrix (48). Future work is warranted to investigate if SUMOylation is enriched at the nuclear matrix and contributes to formation of the nuclear matrix network, given the prevailing role of SUMOylation in mediating protein–protein interaction (5, 18).

Our finding also raises an important question as to how nuclear matrix–associated PIAS proteins can function in the context of chromatin. The identification of multiple histones H3 and H2A.Z as the top PIAS1 direct interacting proteins provides a reasonable explanation to this issue (Fig. 3 and Table S1). In addition, actin, myosin, and plectin were identified as potential PIAS1 direct binding proteins; whether these potential interactions may contribute to PIAS1 nuclear matrix localization remains to be investigated in the future. We validate by both *in vivo* and *in vitro* assays that PIAS1 binds directly to histones H3 and H2A.Z (Figs. 4 and 5). Furthermore, we demonstrate that purified GST-PIAS1 binds recombinant histone octamers and reconstituted mononucleosomes *in vitro* (Fig. 4G), thus providing compelling evidence for a direct interaction between PIAS1 and histones. It is noteworthy that the SAP domain is required for efficient binding of mononucleosomes and chromatin but not histone octamers *in vitro* (Fig. 5, C–F), suggesting that there is an additional domain(s) within PIAS1 that can also interact with histones. This SAP-independent interaction is likely masked once histone octamers are assembled into nucleosomes. Given that the SAP domain can bind DNA (28, 49), at this stage we could not exclude the possibility that binding of DNA also promotes and contributes to the observed PIAS1–histone interaction. However, the facts that histones H3 and H2A.Z were identified as the top two PIAS1 direct interacting proteins and that recombinant histone octamers and mononucleosomes bound GST-PIAS1 to a similar extent strongly argue for a role of PIAS1–histone interaction in targeting SUMOylation to chromatin. As SAP is a common domain in PIAS family proteins, it is tempting to suggest that all PIAS1 family proteins may also interact directly with histones and this interaction may also contribute to their function in chromatin.

We also find that PIAS1 promotes SUMOylation of multiple histones. Although mammalian histone SUMOylation was discovered in 2003 and shown to cause transcriptional repression (13), there have been limited studies since, possibly owing to difficulty in detection of SUMOylation on endogenous histones. In yeast, histone SUMOylation has been more extensively investigated and shown to play important roles in transcriptional regulation and DNA damage response (14, 50), possibly because histone SUMOylation is more readily detectable in yeast. Consistent with our finding that PIAS1 directly binds histones, we show that PIAS1 promotes SUMOylation on both ectopically expressed and endogenous

histones H3 and H2B (Figs. 6 and 7). PIAS1 also promotes SUMOylation of ectopically expressed H2A, although SUMOylation on endogenous H2A was not detected, suggesting that PIAS1 may only weakly promote H2A SUMOylation. PIAS1 does not appear to promote SUMOylation on H4 and H2A.Z, despite a direct interaction between PIAS1 and H2A.Z. It is also noteworthy that H1 linker histones were also identified as PIAS1 direct interacting proteins in our study, and SUMOylation of histone H1 was reported (9), suggesting that PIAS1 may also promote H1 SUMOylation. Although our data clearly support a role of PIAS1 in histone SUMOylation, it is highly likely that PIAS1 also promotes SUMOylation of nonhistone proteins in chromatin.

A previous study indicated that HS-induced genes were markedly repressed by a promoter SUMOylation that was impaired in PIAS1-knockdown cells (21). To test the role of the PIAS1 SAP domain in chromatin association and transcriptional regulation, we re-expressed wildtype and a SAP-deleted PIAS1 mutant in PIAS1-knockout HeLa cells. We found that re-expression of wildtype PIAS1 but not the SAP deletion mutant is able to partially suppress HS-induced activation of several HS-induced genes (Fig. 8, B–E). A partial restoration of repression by re-expression of wildtype PIAS1 is likely due to a less than 100% transfection efficiency (~80%–95%) in the experiments. Subsequent ChIP assay revealed an essential role of the SAP domain in binding to chromatin by PIAS1 (Fig. 8, F–I). Thus, the SAP domain–mediated interaction between PIAS1 and H3/H2A.Z is likely important for PIAS1 to bind chromatin and regulate transcription. Future work is warranted to dissect the function of SAP histone binding observed here from its previously reported DNA binding activity.

Previous genome-wide studies indicate that SUMOylated proteins are enriched at transcriptionally active regions (21–23). In this regard, our finding that PIAS1 binds preferentially histone H2A.Z is interesting, as H2A.Z is known to enrich at the promoters of active genes (44, 45). Thus, it is tempting to propose that PIAS1 could be targeted to actively transcribed genes by its interaction with H2A.Z, where it suppresses transcription by promoting histone and possibly nonhistone SUMOylation.

Experimental procedures

Cell culture and transfection

Human embryonic kidney 293T (HEK293T) cells, human cervical cancer HeLa cells, and primary hepatocytes were maintained in Dulbecco's modified Eagle's medium (GIBCO) containing 10% fetal bovine serum (GIBCO), 100 U/ml penicillin, and 100 µg/ml streptomycin (GIBCO). Primary hepatocytes were isolated as described (51). Cell transfection was performed using Lipofectamine 2000 (Invitrogen) according to manufacturer's instruction.

Antibodies and constructs

The following primary antibodies were used in this study: SUMO1 (AbClonal, A2130), histone H3 (Abcam, ab1791), histone H2A (Abcam, ab18255), histone H2B (Abcam, ab1790),

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histone H4 (Abcam, ab10158), histone H2A.Z (Abcam, ab4174), Lamin A/C (Abcam, ab133256), PIAS1 (Abcam, ab109388), SAE1 (Abcam, ab185552), SAE2 (Abcam, ab185955), UBC9 (homemade), HSF1 (CST, 12972), β -actin (HUABIO, M1210-2), GAPDH (Abmart, M20006L), HA (Abmart, M20003), and FLAG (Sigma, F7425/F1804). For ectopic expression of histones, the DNA sequences encoding H3, H2A, H2B, H4, and H2A.Z were, respectively, subcloned into p3 \times FLAG-CMV-10 vector (Sigma). For expression of PIAS family proteins, the cDNA fragments encoding PIAS1, PIASx α , PIASx β , PIAS3, and PIASy were, respectively, subcloned into a HA tagged pCDNA3.0 vector. GFP-tagged and His-tagged SUMO-1 expression constructs were as described (16). To express PIAS1 as GST fusion protein, the PIAS1 encoding region was PCR amplified and subcloned into PGEX4T-1 vector. The plasmids for pCDNA3.0-PIAS1(1–100), pCDNA3.0-PIAS1(1–300), pCDNA3.0-PIAS1(1–400), and pCDNA3.0-PIAS1- Δ SAP were derived from pCDNA3.0-HA-PIAS1 by site-directed mutagenesis and confirmed by sequencing. pGEX4T1-PIAS1(1–100), pGEX4T1-PIAS1(1–300), pGEX4T1-PIAS1(1–400), and pGEX4T1-PIAS1- Δ SAP were derived from pGEX4T1-PIAS1 by site-directed mutagenesis and confirmed by sequencing. pCDNA3.0-PIAS1m encodes a PIAS1 mutant with C346S, C351S, and C356S mutations as described (16).

DSP cross-linking-based isolation of PIAS1 direct interacting proteins

To isolate and identify PIAS1 direct interacting proteins, HA-PIAS1 plasmids were transfected into HEK293T cells. Two days after transfection, the cells were collected and washed with 1 \times PBS and treated with or without 2 mM DSP (Thermo Scientific, 22585) in 1 \times PBS at room temperature for 30 min. Tris-HCl (pH 7.5) was then added to a final concentration of 20 mM and incubated for 15 min to stop the cross-linking reaction. The cells were collected by centrifugation and lysed in ice-cold hypotonic buffer (10 mM Hepes, pH 7.4, 5 mM MgCl₂, 10 mM NaCl) with 1 \times protease inhibitor cocktail and incubated for 30 min on ice. Then the soluble cytoplasmic fraction was separated from the insoluble nuclear fraction by centrifugation at 3000 rpm for 20 min at 4 °C. The nuclear pellet was washed twice with 1 \times PBS and then resuspended in five volumes of 7 M urea containing 1% Triton X-100 and 1 \times protease inhibitor cocktail and incubated on ice for 20 min. A part of the lysate was saved as input, and the remaining nuclear lysate was diluted eight times with 1 \times PBS containing 1 \times protease inhibitor cocktail and sonicated three times for 15 s each on ice with power setting at 40 W (SCI-ENTZ JY92-IIN). The supernatants were obtained after centrifugation at 12,000 rpm at 4 °C for 20 min and incubated with anti-HA agarose beads with rotation for 4 h at 4 °C. The HA beads were washed three times with Washing Buffer (50 mM Tris-HCl pH7.5, 150 mM NaCl, 1 mM EDTA, 0.1% TritonX-100, and 1 \times protease inhibitor cocktail) and subjected to LC-MS analysis. Alternatively, the HA beads were boiled in 1 \times SDS loading buffer and subjected to Western blot analysis.

For confirmation of direct interaction between PIAS1 and histones upon DSP cross-linking, cells were lysed in RIPA buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% SDS, and 1 \times protease inhibitor cocktail) and the soluble supernatants were used for immunoprecipitation–Western blot analysis.

LC-MS analysis and data analysis

For LC-MS analysis, the protein-bound beads were washed six times with ice-cold PBS and incubated at room temperature for 15 min in 30 μ l of 8 M urea. Proteins were reduced with 10 mM DTT for 30 min at 55 °C, then alkylated with 20 mM iodoacetamide for 30 min in dark. A volume of 120 μ l of 50 mM ammonium bicarbonate was added to dilute the concentration of urea, and the proteins were digested with 0.6 μ g trypsin at 37 °C overnight. After digestion, peptides were desalted with reverse phase C18 SPE disk and eluted with 60% of acetonitrile containing 0.1% formic acid. Desalted peptides were vacuum dried before LC-MS analysis. The LC-MS analysis was performed using an EASY-nL 1000 system coupled online to a Q-Exactive mass spectrometer (Thermo Scientific). Briefly, aliquots from two samples containing 1 to 2 μ g of peptides were injected and separated on a reversed-phase column (15 cm \times 70 μ m, 3 μ m) using a 1-h gradient from 12% to 32% in buffer B (98% acetonitrile, 0.1% formic acid) at a flow rate of 250 nl/min. The MS data were acquired using data-dependent method at positive mode. MS1 spectra of precursors ranging from 300 to 1600 *m/z* were collected at 70,000 resolution with 100 ms maximum injection time and 3e6 automatic gain control target value. The top 12 most intense precursors were selected for MS2 analysis at a resolution of 17,500, the automatic gain control target value was set as 5e5, and the maximum injection time was set as 60 ms. A 2-*m/z* isolation window and 27 normalized collision energy were used to product fragment ions. Precursors with charge states 2 to 7 were selected for fragmentation and 15-s dynamic exclusion was applied.

The MS raw files were searched against the uniprot human reviewed protein database (42,289 entries, downloaded on November 14, 2020) using Sequest HT algorithm embedded in Proteome Discover Software (Thermo Scientific, version: 1.4.1.14). Spectrum Selector embedded in Proteome Discover Software was used to generate peak list. Trypsin was the specific protease used to generate peptides, and no more than two missed cleavages were allowed. Carbamidomethyl of cystine (+57.021 Da) was considered as fixed modification; oxidation of methionine (+15.995 Da) and DSP cross-linking of lysine (+146.019 Da) were considered as variable modifications. The mass tolerance of precursor and product ions were 10 ppm and 0.02 Da, respectively. The target decoy PSM validator method was used to determine the false discovery rate, and 0.01 was set as the strict false discovery rate. Only high-confidence peptides were included in final output result. The overlap between two experimental groups was calculated and presented as a Venn diagram. Gene ontology enrichment was performed using DAVID 6.8 website resource.

Purification of GST fusion proteins

pGEX4T1-PIAS1 was transformed into *E. coli* Rosetta. A single colony was inoculated in LB medium with 100 µg/ml ampicillin and cultured at 37 °C until the culture reached exponential phase. Expression of GST fusion proteins was induced by 100 mM IPTG (isopropyl β-D-1-thiogalactopyranoside) for 10 h at 16 °C. The cells were pelleted and lysed by sonication in Buffer A (1 × PBS buffer containing 10% glycerol, 1 × protease inhibitor cocktail, and 1 mM DTT). After centrifugation at 10,000 rpm for 15 min, the supernatants were incubated with Glutathione Sepharose beads (GenScript) for 4 h at 4 °C. The GST fusion protein-bound beads were washed three times with cold Washing Buffer (1 × PBS buffer containing 0.05% Triton X-100) and were ready for *in vitro* pulldown experiments.

GST pulldown assay

For GST pulldown of ectopically expressed and endogenous histones, the cells expressing FLAG-H3 or FLAG-H2A.Z were lysed in buffer containing 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 8% glycerol, 2 mM EDTA, 1 × Protease Inhibitor cocktail, and 1 mM DTT and sonicated to solubilize chromatin. After centrifugation at 12,000 rpm at 4 °C for 20 min, the supernatants were diluted 1-fold with lysis buffer without Triton X-100 and incubated with GST-, GST-PIAS1-, or GST-PIAS1 mutant-bound Glutathione Sepharose beads at 4 °C for 4 h on a rotator. Recombinant histone octamers or mononucleosomes were prepared as described (52) and pulldown was performed in binding buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 8% glycerol, 0.1% Triton X-100, 2 mM EDTA, 1 × protease inhibitor cocktail, 1 mM DTT, and 0.1 µg/µl bovine serum albumin). The GST beads were washed three times with Washing Buffer (20 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1 mM EDTA, 0.05% Triton X-100, 1 × protease inhibitor cocktail, and 1 mM DTT) and subjected for Western blot analysis.

Subcellular fractionation

To separate cell extracts into cytosol, soluble nuclear extract and chromatin plus nuclear matrix fractions, cells were washed with cold 1 × PBS twice. Cells were resuspended with 250 µl Buffer A (10 mM Hepes-NaOH, pH 7.9, 10 mM KCl, 1.5 mM MgCl₂, and 0.5 mM β-Mercaptoethanol) and incubated on ice for 15 to 20 min. NP-40 was added to a final concentration of 0.1%, vortexed, and incubated on ice for 10 min. The samples were centrifuged at 12,000 rpm for 10 to 15 min, and the resulting supernatants were designated as cytosols. The nuclear pellets were washed with cold 1 × PBS twice, then were resuspended with 60 to 80 µl Buffer B (10 mM Tris-HCl, pH 7.6, 420 mM NaCl, 0.5% NP-40, 2 mM MgCl₂ plus 1 × protease inhibitor cocktail, 50 mM N-Ethylmaleimide and 1 mM DTT). The mixtures were incubated on ice for 20 min and centrifuged at 12,000 rpm for 10 to 15 min. The supernatants were designated as nuclear extracts, whereas the pellets

washed with Buffer B once were designated as the chromatin plus nuclear matrix.

Preparation of the nuclear matrix fraction

Preparation of nuclear matrix by removal of chromatin with nuclease digestion and high salt extraction was carried out essentially as described (40). Briefly, cells were collected after wash in PBS and lysed in CSK Buffer (10 mM Pipes, pH 6.8, 100 mM NaCl, 300 mM Sucrose, 3 mM MgCl₂, 1 mM EGTA, supplemented with 1 × protease inhibitor cocktail, 50 mM N-Ethylmaleimide, 1 mM DTT, and 0.5% Triton X-100). After 10 min incubation on ice, the pellets with chromatin plus the nuclear matrix were separated from soluble proteins by centrifugation at 5000g for 3 min. The pellets were resuspended in CSK buffer and chromatin was digested with 5 units of RNase-free DNase I (Promega) for 30 min or Benzonase for time as indicated at 37 °C. Then, ammonium sulfate was added in CSK buffer to a final concentration of 0.25 M and samples were pelleted again after 5 min incubation on ice. The pellets were further extracted with 2 M NaCl (1 × protease inhibitor cocktail, 50 mM N-Ethylmaleimide, 1 mM DTT) in CSK buffer for 15 min on ice and then centrifuged. This treatment removed all the DNA and the histones from nuclear pellets. The remaining pellets were designated as nuclear matrix and solubilized in Urea buffer (8 M urea, 0.1 M NaH₂PO₄, 0.01 M Tris, pH 8, 1 × protease inhibitor cocktail, 50 mM N-Ethylmaleimide, and 1 mM DTT).

Immunofluorescence analysis of the nuclear matrix-associated proteins

Immunofluorescence assay of the nuclear matrix-associated proteins was performed as described (53). In brief, HeLa cells grown on cover slips were washed with cold 1 × PBS twice and permeabilized with 0.5% Triton X-100 in CSK Buffer (100 mM NaCl, 300 mM Sucrose, 10 mM Pipes, pH 6.8 and 5 mM MgCl₂) for 10 min at room temperature. After washing twice with CSK with 1 × protease inhibitor cocktail, cells were incubated with 50 or 250 U Benzonase (Merck, 71205-3) in CSK with 1 × protease inhibitor cocktail for times as indicated at 37 °C. After two washes with CSK buffer, cells were fixed with 4% paraformaldehyde for 20 min at 4 °C, washed with cold 1 × PBS twice and then permeabilized again with 1% Triton X-100 in 1 × PBS for 20 min on ice. After washing with cold 1 × PBS twice, the cells were blocked with 5% bovine serum albumin in 0.2% PBST (0.2% Triton X-100 in PBS) for 1 h at 37 °C, washed with cold 1 × PBS twice, and incubated with primary antibody overnight at 4 °C followed by incubation with fluorescently conjugated secondary antibodies for 1 h at room temperature. The images were acquired with a confocal microscope (Carl Zeiss).

SUMOylation assays by Ni²⁺-NTA pulldown

To detect SUMOylation on endogenous histones, HEK293T cells were transfected with His-SUMO1 and wildtype or mutant PIAS1 as indicated. The SUMOylated proteins were purified under denatured condition using Ni²⁺-NTA beads as

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described (54), and histone SUMOylation was detected by Western blot analysis using antibodies as indicated.

Heat shock treatment and quantitative RT-qPCR

Heat shock treatment was done by moving tissue culture plates with growing cells from an incubator at normal growth temperature (37 °C) into an incubator at heat-shock temperatures (42 °C) for 40 min. For RT-qPCR analysis, total RNAs were extracted from cells using the Total Trizol extraction kit from TOROIVD (A211-01). cDNAs were synthesized using the reverse transcription kit TOYOBO (FSQ-301). Subsequent quantitative PCRs were performed using the TOROGreen qPCR Master Mix (TOROIVD, QST-100) on the QuantStudio 3 Real-Time PCR System (Applied Biosystems) using the comparative Ct quantitation method. Results were normalized to GAPDH mRNA levels. The PCR protocol was as follows: initial denaturing at 95 °C for 5 min, 40 cycles of 95 °C for 20 s, 60 °C for 30 s, and 72 °C for 30 s, followed by a final extension at 72 °C for 5 min. Melt curve analysis was performed on a QuantStudio 3 Real-Time PCR System (Applied Biosystems) coupled with QuantStudio Design & Analysis Software. The primer sequences used in the RT-qPCR are listed in Table S2.

Chromatin immunoprecipitation assay

ChIP was performed with anti-HA essentially as described (55).

Statistical analysis

All statistical analyses were performed *via* GraphPad Prism 5.0 software. The data are presented as mean of three independent experiments. Statistical relevance was determined using the unpaired Student's test for RT-qPCR. The error bars represent \pm SD. Differences of $p < 0.05$ were considered significant. In all results, “*” , $0.01 < p < 0.05$; “**” , $p < 0.01$; “***” , $p < 0.001$; and “****” , $p < 0.0001$.

Data availability

All the data supporting our conclusions are presented in this article and the Figs. S1–S3 and Tables S1 and S2. All material is available upon request. The raw mass spectrometry data, searching output msf format files, protein identification excel tables, and annotated one peptide spectra are deposited in a publicly accessible repository Zenodo. The DOI is 10.5281/zenodo.5227669.

Supporting information—This article contains [supporting information](#).

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Author contributions—Z. C., J. W., and Jiwen Li conceptualization; Z. C., Y. Z., Jialun Li, and W. W. methodology; Q. G., H. Z., and X. X. validation; Y. Z. formal analysis; Z. C., Y. Z., and Jing Luo investigation; Z. C. writing – original draft; J. W. and Jiwen Li writing – review & editing; Z. C. visualization; L. L., J. W., and Jiwen Li supervision; Jiwen Li project administration; J. W. and Jiwen Li funding acquisition.

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Conflicts of interest—The authors declare that they have no conflicts of interest with the contents of this article.

Abbreviations—The abbreviations used are: ChIP, chromatin immunoprecipitation; co-IP, coimmunoprecipitation; DSP, dithio-bis succinimidyl propionate; SUMO, small ubiquitin-related modifier.

References

1. Cubenas-Potts, C., and Matunis, M. J. (2013) SUMO: A multifaceted modifier of chromatin structure and function. *Dev. Cell* **24**, 1–12
2. Flotho, A., and Melchior, F. (2013) Sumoylation: A regulatory protein modification in health and disease. *Annu. Rev. Biochem.* **82**, 357–385
3. Dhingra, N., and Zhao, X. (2019) Intricate SUMO-based control of the homologous recombination machinery. *Genes Dev.* **33**, 1346–1354
4. Sarangi, P., and Zhao, X. (2015) SUMO-mediated regulation of DNA damage repair and responses. *Trends Biochem. Sci.* **40**, 233–242
5. Zhao, X. (2018) SUMO-mediated regulation of nuclear functions and signaling processes. *Mol. Cell* **71**, 409–418
6. Wu, C. S., Ouyang, J., Mori, E., Nguyen, H. D., Marechal, A., Hallet, A., Chen, D. J., and Zou, L. (2014) SUMOylation of ATRIP potentiates DNA damage signaling by boosting multiple protein interactions in the ATR pathway. *Genes Dev.* **28**, 1472–1484
7. Varejao, N., Lascorz, J., Li, Y., and Reverter, D. (2020) Molecular mechanisms in SUMO conjugation. *Biochem. Soc. Trans.* **48**, 123–135
8. Hendriks, I. A., D'Souza, R. C., Yang, B., Verlaan-de Vries, M., Mann, M., and Vertegaal, A. C. (2014) Uncovering global SUMOylation signaling networks in a site-specific manner. *Nat. Struct. Mol. Biol.* **21**, 927–936
9. Matafora, V., D'Amato, A., Mori, S., Blasi, F., and Bachi, A. (2009) Proteomics analysis of nucleolar SUMO-1 target proteins upon proteasome inhibition. *Mol. Cell. Proteomics* **8**, 2243–2255
10. Li, C., McManus, F. P., Plutoni, C., Pascariu, C. M., Nelson, T., Alberici Delsin, L. E., Emery, G., and Thibault, P. (2020) Quantitative SUMO proteomics identifies PIAS1 substrates involved in cell migration and motility. *Nat. Commun.* **11**, 834
11. Lumpkin, R. J., Gu, H., Zhu, Y., Leonard, M., Ahmad, A. S., Clauser, K. R., Meyer, J. G., Bennett, E. J., and Komives, E. A. (2017) Site-specific identification and quantitation of endogenous SUMO modifications under native conditions. *Nat. Commun.* **8**, 1171
12. Hendriks, I. A., and Vertegaal, A. C. (2016) A comprehensive compilation of SUMO proteomics. *Nat. Rev. Mol. Cell Biol.* **17**, 581–595
13. Shiio, Y., and Eisenman, R. N. (2003) Histone sumoylation is associated with transcriptional repression. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 13225–13230
14. Nathan, D., Ingvarsdottir, K., Sterner, D. E., Bylebyl, G. R., Dokmanovic, M., Dorsey, J. A., Whelan, K. A., Krsmanovic, M., Lane, W. S., Meluh, P. B., Johnson, E. S., and Berger, S. L. (2006) Histone sumoylation is a negative regulator in *Saccharomyces cerevisiae* and shows dynamic interplay with positive-acting histone modifications. *Genes Dev.* **20**, 966–976
15. Ryu, H. Y., Zhao, D., Li, J., Su, D., and Hochstrasser, M. (2020) Histone sumoylation promotes Set3 histone-deacetylase complex-mediated transcriptional regulation. *Nucleic Acids Res.* **48**, 12151–12168

16. Yu, F., Shi, G., Cheng, S., Chen, J., Wu, S. Y., Wang, Z., Xia, N., Zhai, Y., Wang, Z., Peng, Y., Wang, D., Du, J. X., Liao, L., Duan, S. Z., Shi, T., *et al.* (2018) SUMO suppresses and MYC amplifies transcription globally by regulating CDK9 sumoylation. *Cell Res.* **28**, 670–685
17. Peng, Y., Wang, Z., Wang, Z., Yu, F., Li, J., and Wong, J. (2019) SUMOylation down-regulates rDNA transcription by repressing expression of upstream-binding factor and proto-oncogene c-Myc. *J. Biol. Chem.* **294**, 19155–19166
18. Hay, R. T. (2013) Decoding the SUMO signal. *Biochem. Soc. Trans.* **41**, 463–473
19. Huang, J., Yan, J., Zhang, J., Zhu, S., Wang, Y., Shi, T., Zhu, C., Chen, C., Liu, X., Cheng, J., Mustelin, T., Feng, G. S., Chen, G., and Yu, J. (2012) SUMO1 modification of PTEN regulates tumorigenesis by controlling its association with the plasma membrane. *Nat. Commun.* **3**, 911
20. Lin, X., Liang, M., Liang, Y. Y., Brunicardi, F. C., and Feng, X. H. (2003) SUMO-1/Ubc9 promotes nuclear accumulation and metabolic stability of tumor suppressor Smad4. *J. Biol. Chem.* **278**, 31043–31048
21. Niskanen, E. A., Malinen, M., Sutinen, P., Toropainen, S., Paakinaho, V., Vihervaara, A., Joutsen, J., Kaikkonen, M. U., Sistonen, L., and Palvimo, J. J. (2015) Global SUMOylation on active chromatin is an acute heat stress response restricting transcription. *Genome Biol.* **16**, 153
22. Neyret-Kahn, H., Benhamed, M., Ye, T., Le Gras, S., Cossec, J. C., Lapaquette, P., Bischof, O., Ouspenskaia, M., Dasso, M., Seeler, J., Davidson, I., and Dejean, A. (2013) Sumoylation at chromatin governs coordinated repression of a transcriptional program essential for cell growth and proliferation. *Genome Res.* **23**, 1563–1579
23. Toropainen, S., Malinen, M., Kaikkonen, S., Rytinki, M., Jaaskelainen, T., Sahu, B., Janne, O. A., and Palvimo, J. J. (2015) SUMO ligase PIAS1 functions as a target gene selective androgen receptor coregulator on prostate cancer cell chromatin. *Nucleic Acids Res.* **43**, 848–861
24. Gill, G. (2004) SUMO and ubiquitin in the nucleus: Different functions, similar mechanisms? *Genes Dev.* **18**, 2046–2059
25. Girdwood, D. W., Tatham, M. H., and Hay, R. T. (2004) SUMO and transcriptional regulation. *Semin. Cell Dev. Biol.* **15**, 201–210
26. Rabellino, A., Andreani, C., and Scaglioni, P. P. (2017) The role of PIAS SUMO E3-ligases in cancer. *Cancer Res.* **77**, 1542–1547
27. Rytinki, M. M., Kaikkonen, S., Pehkonen, P., Jaaskelainen, T., and Palvimo, J. J. (2009) PIAS proteins: Pleiotropic interactors associated with SUMO. *Cell. Mol. Life Sci.* **66**, 3029–3041
28. Okubo, S., Hara, F., Tsuchida, Y., Shimotakahara, S., Suzuki, S., Hatanaka, H., Yokoyama, S., Tanaka, H., Yasuda, H., and Shindo, H. (2004) NMR structure of the N-terminal domain of SUMO ligase PIAS1 and its interaction with tumor suppressor p53 and A/T-rich DNA oligomers. *J. Biol. Chem.* **279**, 31455–31461
29. Kahyo, T., Nishida, T., and Yasuda, H. (2001) Involvement of PIAS1 in the sumoylation of tumor suppressor p53. *Mol. Cell* **8**, 713–718
30. Liu, B., Liao, J., Rao, X., Kushner, S. A., Chung, C. D., Chang, D. D., and Shuai, K. (1998) Inhibition of Stat1-mediated gene activation by PIAS1. *Proc. Natl. Acad. Sci. U. S. A.* **95**, 10626–10631
31. Nishida, T., and Yasuda, H. (2002) PIAS1 and PIASxalpha function as SUMO-E3 ligases toward androgen receptor and repress androgen receptor-dependent transcription. *J. Biol. Chem.* **277**, 41311–41317
32. Galanty, Y., Belotserkovskaya, R., Coates, J., Polo, S., Miller, K. M., and Jackson, S. P. (2009) Mammalian SUMO E3-ligases PIAS1 and PIAS4 promote responses to DNA double-strand breaks. *Nature* **462**, 935–939
33. Rabellino, A., Carter, B., Konstantinidou, G., Wu, S. Y., Rimessi, A., Byers, L. A., Heymach, J. V., Girard, L., Chiang, C. M., Teruya-Feldstein, J., and Scaglioni, P. P. (2012) The SUMO E3-ligase PIAS1 regulates the tumor suppressor PML and its oncogenic counterpart PML-RARA. *Cancer Res.* **72**, 2275–2284
34. Yang, S. H., and Sharrocks, A. D. (2004) SUMO promotes HDAC-mediated transcriptional repression. *Mol. Cell* **13**, 611–617
35. Sachdev, S., Bruhn, L., Sieber, H., Pichler, A., Melchior, F., and Groschedl, R. (2001) PIASy, a nuclear matrix-associated SUMO E3 ligase, represses LEF1 activity by sequestration into nuclear bodies. *Genes Dev.* **15**, 3088–3103
36. Zhou, S., Si, J., Liu, T., and DeWille, J. W. (2008) PIASy represses CCAAT/enhancer-binding protein delta (C/EBPdelta) transcriptional activity by sequestering C/EBPdelta to the nuclear periphery. *J. Biol. Chem.* **283**, 20137–20148
37. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res.* **11**, 1475–1489
38. Sureka, R., Wadhwa, R., Thakur, S. S., Pathak, R. U., and Mishra, R. K. (2018) Comparison of nuclear matrix and mitotic chromosome scaffold proteins in Drosophila S2 cells-transmission of hallmarks of nuclear organization through mitosis. *Mol. Cell. Proteomics* **17**, 1965–1978
39. Pathak, R. U., Rangaraj, N., Kallappagoudar, S., Mishra, K., and Mishra, R. K. (2007) Boundary element-associated factor 32B connects chromatin domains to the nuclear matrix. *Mol. Cell. Biol.* **27**, 4796–4806
40. Reyes, J. C., Muchardt, C., and Yaniv, M. (1997) Components of the human SWI/SNF complex are enriched in active chromatin and are associated with the nuclear matrix. *J. Cell Biol.* **137**, 263–274
41. Jackson, S. P., and Durocher, D. (2013) Regulation of DNA damage responses by ubiquitin and SUMO. *Mol. Cell* **49**, 795–807
42. Maheswaran, S., Lee, H., and Sonenshein, G. E. (1994) Intracellular association of the protein product of the c-myc oncogene with the TATA-binding protein. *Mol. Cell. Biol.* **14**, 1147–1152
43. Fu, J., Jiang, J., Li, J., Wang, S., Shi, G., Feng, Q., White, E., Qin, J., and Wong, J. (2009) Deleted in breast cancer 1, a novel androgen receptor (AR) coactivator that promotes AR DNA-binding activity. *J. Biol. Chem.* **284**, 6832–6840
44. Gaiamo, B. D., Ferrante, F., Herchenrother, A., Hake, S. B., and Borggreve, T. (2019) The histone variant H2A.Z in gene regulation. *Epigenetics Chromatin* **12**, 37
45. Subramanian, V., Fields, P. A., and Boyer, L. A. (2015) H2A.Z: A molecular rheostat for transcriptional control. *F1000Prime Rep.* **7**, 01
46. Albrethsen, J., Knol, J. C., and Jimenez, C. R. (2009) Unravelling the nuclear matrix proteome. *J. Proteomics* **72**, 71–81
47. Razin, S. V., Iarovaia, O. V., and Vassetzky, Y. S. (2014) A requiem to the nuclear matrix: From a controversial concept to 3D organization of the nucleus. *Chromosoma* **123**, 217–224
48. Lallemand-Breitenbach, V., and de Thé, H. (2010) PML nuclear bodies. *Cold Spring Harb. Perspect. Biol.* **2**, a000661
49. Tan, J. A., Song, J., Chen, Y., and Durrin, L. K. (2010) Phosphorylation-dependent interaction of SATB1 and PIAS1 directs SUMO-regulated caspase cleavage of SATB1. *Mol. Cell. Biol.* **30**, 2823–2836
50. Jalal, D., Chalissery, J., and Hassan, A. H. (2017) Genome maintenance in *Saccharomyces cerevisiae*: The role of SUMO and SUMO-targeted ubiquitin ligases. *Nucleic Acids Res.* **45**, 2242–2261
51. Koh, A., Molinaro, A., Stahlman, M., Khan, M. T., Schmidt, C., Manneras-Holm, L., Wu, H., Carreras, A., Jeong, H., Olofsson, L. E., Bergh, P. O., Gerdes, V., Hartstra, A., de Brauw, M., Perkins, R., *et al.* (2018) Microbially produced imidazole propionate impairs insulin signaling through mTORC1. *Cell* **175**, 947–961.e17
52. Zhao, Q., Zhang, J., Chen, R., Wang, L., Li, B., Cheng, H., Duan, X., Zhu, H., Wei, W., Li, J., Wu, Q., Han, J. D., Yu, W., Gao, S., Li, G., *et al.* (2016) Dissecting the precise role of H3K9 methylation in crosstalk with DNA maintenance methylation in mammals. *Nat. Commun.* **7**, 12464
53. Abad, P. C., Lewis, J., Mian, I. S., Knowles, D. W., Sturgis, J., Badve, S., Xie, J., and Lelievre, S. A. (2007) NuMA influences higher order chromatin organization in human mammary epithelium. *Mol. Biol. Cell* **18**, 348–361
54. Li, R., Wei, J., Jiang, C., Liu, D., Deng, L., Zhang, K., and Wang, P. (2013) Akt SUMOylation regulates cell proliferation and tumorigenesis. *Cancer Res.* **73**, 5742–5753
55. Schmidt, D., Wilson, M. D., Spyrou, C., Brown, G. D., Hadfield, J., and Odom, D. T. (2009) ChIP-seq: Using high-throughput sequencing to discover protein-DNA interactions. *Methods* **48**, 240–248