RING tetramerization is required for nuclear body biogenesis and PML sumoylation

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ProMyelocyticLeukemia nuclear bodies (PML NBs) are stress-regulated domains directly implicated in acute promyelocytic leukemia eradication. Most TRIM family members bind ubiquitin E2s and many acquire ligase activity upon RING dimerization. In contrast, PML binds UBC9, the SUMO E2 enzyme. Here, using X-ray crystallography and SAXS characterization, we demonstrate that PML RING tetramerizes through highly conserved PML-specific sequences, which are required for NB assembly and PML sumoylation. Conserved residues implicated in RING dimerization of other TRIMs also contribute to PML tetramer stability. Wild-type PML rescues the ability of some RING mutants to form NBs as well as their sumoylation. Impaired RING tetramerization abolishes PML/RARA-driven leukemogenesis in vivo and arsenic-induced differentiation ex vivo. Our studies thus identify RING tetramerization as a key step in the NB macro-molecular scaffolding. They suggest that higher order RING interactions allow efficient UBC9 recruitment and thus change the biochemical nature of TRIM-facilitated post-translational modifications.
PML nuclear bodies (NBs) are membrane-less insoluble structures whose assembly increases upon stress and which recruit a large number of partner proteins. PML NBs also recruit enzymes implicated in several post-translational modifications, primarily UBC9, the key E2 SUMO-conjugating enzyme, possibly facilitating partner sumoylation. There is evidence that PML NBs regulate stress responses, in particular senescence induction, at least in part through the control of p53 activation. Indeed, NBs are directly implicated in the eradication of acute promyelocytic leukemia (APL) by retinoic acid and arsenic therapy, notably by inducing p53-mediated senescence. PML belongs to the TRIM family of proteins, defined by the presence of a RING domain, one or two other zinc domains (see below). The interactions between SD1FQF/SD2 and across each face (Supplementary Figure 1a) of PML RING, B boxes and coiled coil are all important for NB-biogenesis. Although PML sumoylation was proposed to drive PML NB-biogenesis, SUMO is required for NB client protein recruitment rather than PML self-assembly into NBs.

Here we report the crystallographic high-resolution structure of PML RING, and demonstrate that it assembles into a tetrameric torus. Interfaces of these tetramers involve PML-specific sequences that are highly conserved during evolution. Critically, their mutation prevents PML tetramerization in solution and abolishes NB formation and PML sumoylation in cellulo. Our data suggest that this novel macro-molecular RING assembly may control interactions with E2s and hence TRIM functions.

Results
RING crystal structure reveals a torus-shaped tetramer.
We have determined the crystal structure of PML RING at a 1.6 Å resolution by single wavelength anomalous dispersion (Fig. 1a, Supplementary Figure 1a–e). Similar to previous RING structures, the folding of PML RING is coordinated by two Zn ions, but in contrast with previous studies, PML RING leads to tetrameric complexes. Within a tetramer, each PML subunit adopts a donut shape configuration with two distinct subdomains. Sub-domain 1 (SD1EFG) is a loop containing only three residues, F52Q53F54, while sub-domain 2 (SD2), comprising residues 55–99, is a classic C_HC_R RING finger (Fig. 1a,b). Together with homo-dimerization of SD2, interactions between SD1EFG and SD2 create a four subunits torus-like structure (Fig. 1c). An extended PML sequence, while the N-terminal sequence is not found in PML RING, the highly conserved C-terminal amino acids are present in PML, with N106 and L122 being the two most conserved residues in this TRIM-specific helix (Fig. 1a). Together the residues involved in both interfaces are not only highly conserved among PML orthologs (purple boxes in Fig. 1b), but also highly specific to PML within the TRIM family. The RING tetramer harbors four highly charged patches at each side of the torus as well as a symmetrical deep groove diagonally across each face (Supplementary Figure 1a–e), which could constitute binding sites for partner proteins and/or other PML domains (see below).

The interactions between SD1EFG/SD2 and SD2/SD2 interfaces of the PML RING tetramer are mediated by: (i) the interaction between F52 from SD1EFG loop of subunit 1, and the hydrophobic pocket delineated by the side chains of L70, L81, W95 from SD2 of subunit 2 (Fig. 1c); (ii) the hydrophobic interactions between F54 from SD1EFG, K65 from SD2 of the same subunit 1 and K68 from SD2 of subunit 2, so that the benzyl side chain of F54 is sandwiched by the side chains of K65 and K68 (Fig. 1c); (iii) an intermolecular disulfide bridge C66-C66 in the crystal; (iv) two adjacent SD2 subunits pack against each other in a face-to-face configuration mainly mediated by the L73-L73 hand-shake-like hydrophobic interaction, but also by a highly evolutionarily conserved loop around C91 (Fig. 1a,b,d).

When the previous NMR RING structure is superimposed with the crystallographic one, significant differences appear (Fig. 1e). In the previously described monomeric structure, SD1EFG was proposed to interact with the SD2 of the same subunit. Within the current tetramer, the N-terminal F52Q53F54 SD1EFG undergoes a radical 23 Å swing away from the adjacent SD2, allowing the SD1EFG loop to engage with the hydrophobic pocket of the other subunit. This shift also exposes L73 and allows SD2 dimerization (Fig. 1e). As estimated by the AREAIMOL program, the buried surfaces between one set of SD1EFG/SD2 and SD2/SD2 dimers are 1333 and 779 Å², respectively (Fig. 1f).

As oligomerization proceeds, the buried surface of PML RING tetramer increases significantly to 4216 Å², accounting for ~40% of the overall surface of PML RING tetramer (Fig. 1f).

Tetramerization in solution through highly conserved sequences.
Existence of PML RING tetramers is supported by biochemical analyses: analytical ultracentrifugation experiments of PML RING9–104 showed three peaks corresponding to molecular masses of 7.5, 15 and 34 kDa, consistent with RING monomer, dimer and tetramer formation (Fig. 2a). Importantly, disruption of either interfaces abolished complex formation (Fig. 2b), while not altering the RING overall fold (Supplementary Figure 2). In order to further document PML RING tetramerization in solution, we used small-angle-X-ray scattering (SAXS) (Fig. 2c). The match in crystal fitting with a χ² value of 1.19 suggested the existence of PML RING monomer (60.1%), dimer (26.1%) and tetramer (13.8%) in solution (Fig. 2d). We then performed gel filtration of PML deleted for B boxes and coiled coil domains (ABC) to prevent oligomerization, with or without F52/S4E or L73E, or with mutations of the SD1EFG-interacting amino acid K65, K68 and L81 in SD2 (Supplementary Figure 4a). These PML mutants were consistently found in lower molecular weight fractions compared to PMLABC, suggesting that they lost their self-interaction properties. Similarly, when the F52/S4E mutant was mixed with PMLABC, the latter was found in lower molecular weight fractions, suggestive for impairment of its tetramerization (Supplementary Figure 4b).

Tetramers are also stabilized by TRIM-conserved sequence.
Other TRIM RING domains form dimers rather than tetramers, prompting a detailed analysis of the evolutionary diversity between PML- and TRIM- RING sequences (Fig. 3a and Supplementary Figure 3). Among TRIM RINGs, interactions between N-terminal and C-terminal helix segments were recently implicated in TRIM5, TRIM25 or TRIM32 dimer formation. While the N-terminal sequence is not found in PML RING, the highly conserved C-terminal amino acids are present in PML, with N106 and L122 being the two most conserved residues in this TRIM-specific helix (Fig. 3a). An extended PML sequence, RING1–119 was thus subjected to gel filtration and SEC-MALS analysis. Critically, we obtained evidence for stable tetrameric and dimeric assembly in solution (Fig. 3b–e). Mutations of either of N106R or L112R inhibited PML RING tetramerization (Fig. 3f).

Critically, mutations of residues implicated in the tetramer interfaces (F52Q53F54 and L73), which do not alter the RING overall fold (Supplementary Figure 2), also precluded tetramerization of the extended RING (Fig. 3f and Supplementary Figure 4). We finally subjected the long version of PML RING to limited proteolysis and mass sequencing, revealing that residues 53–98 constitute its structured core domain (Fig. 3g and Supplementary Figure 5). Remarkably, this domain is almost identical to the one...
**Fig. 1** Crystal structure of PML RING tetramer. 

- **a** Crystal structure of PML RING tetramer. The residues 51–97 of crystallized PML RING49–104 are visible in the electron density map. Four PML monomers are colored in green, magenta, blue and yellow, respectively. The contact residues (F52, F54 and L73) are shown in stick representation. Zn ions are shown in sphere representation. Sub-domain 1 (SD1) and sub-domain 2 (SD2) are bracketed.

- **b** Sequence alignment of the PML RING domains from different species. The conserved residues lying in the F52/54-interfaces and L73-interfaces are highlighted in red, while the conserved Zn-binding residues are colored in cyan. The deep purple boxes underneath the sequences are used to highlight the conserved (greater than 5 out of 6) residues among PML RING.

- **c** Enlarged views of PML RING dimeric interfaces. The residues involving PML oligomerization are shown in stick representation.

- **d** Structural superimposition between different PML RINGS. The NMR and crystallographic PML RINGs are colored in yellow and green, respectively. The L73 positions are labeled with “Asterisk”. The internal Zn-Zn distances and the putative F52Q53F54 swing are highlighted with dash lines.

- **e** The buried areas of the SD1FQF/SD2 and SD2/SD2 interfaces are shown in gray.
used for PML RING crystallization, suggesting that the C-terminal helix bundle may somehow be dissociated from the RING core. Collectively, these biochemical studies support the importance of the RING tetramer interfaces for higher order PML RING interactions.

**NB assembly and PML sumoylation requires RING tetramerization.** To test whether PML RING tetramerization is also important in cellulo, we explored NBs formation and PML sumoylation by stable expression of the mutants in immortalized Pml−/− fibroblasts (Fig. 4). Critically, these mutants generated significantly fewer NBs (PMLF52/54E) or even none (PMLL73E), while the diffuse nuclear staining was dramatically increased. The PMLL73E mutant behaved as PMLF52/54E. The effects of single F to E mutations were less drastic, but nevertheless significantly decreased the numbers of NBs (Supplementary Figure 6a). Similarly, combining mutations of the residues facing F52 and F54 on SD2 (K65/68A-L81E and K65/68A-W95E) also reduced NB formation and increased the nuclear diffuse fraction of PML (Supplementary Figure 6d). In contrast, mutations of C66, which is not evolutionarily conserved (Fig. 1b), to S or A had modest or no effect on NB biogenesis or basal sumoylation (Supplementary Figure 6b,c). This difference in NB re-assembly suggests that the two L73-L73 interactions are required to stabilize the tetrameric torus, while fewer than four SD1FQF/SD2 interfaces may be sufficient. PML RING interacts with UBC9 and PML NBs efficiently recruit UBC9, especially under arsenic-induced oxidative stress2,15 (Fig. 5c). Critically, in our trans-complementation assays, CFP-WT PML co-expression restores efficient basal or arsenic-enhanced sumoylation of HA-PMLF52/54E, but not that of HA-PMLL73E which remained diffusely distributed despite a few aggregates (Fig. 5a and Supplementary Figure 7a). This difference in NB re-assembly suggests that the two L73-L73 interactions are required to stabilize the tetrameric torus, while fewer than four SD1FQF/SD2 interfaces may be sufficient. 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**Fig. 3** Biochemical evidence for RING tetramer formation. 

**a** Consensus sequence among TRIM RINGs (Top). PML conserved sequences are highlighted with purple boxes (Bottom). The conserved TRIM dimeric interface is highlighted with a red line. The Asn and Ile/Leu residues (red arrows) mediate dimeric assembly of other TRIMs.

**b** Gel filtration analysis of recombinant PML RING1–119. Two elution peaks and a last fraction, designated as Peak 1, 2 and fraction 3, respectively. c–e SEC-MALS reanalysis of Peak 1 (c), Peak 2 (d) and fraction 3 (e) derived from previous gel filtration (b). Black curve, the elution profile in UV. Orange curve, the estimated molecular weight. The theoretical molecular weight of PML RING1–119 is 13.1 kDa. Peak 1 sample displayed distinct peaks that correspond to the tetramer (54 kDa), dimer and monomer in the solution. Peak 2 and fraction 3 samples primarily exhibit RING dimer (24 kDa).

**f** Gel filtration of WT RING1–119 and its mutants. g Recombinant PML RING1–119 was subjected to chymotrypsin digestion (Supplementary Figure 5). Arrows indicate the chymotrypsin sites and amino acids indicated in red correspond to the protected fragment, as determined by mass spectrometry and N-terminal sequencing (Box). RING49–104 sequences analyzed by X-ray crystallography are underlined with a blue line.
sumoylation of either PML/RARA<sup>F52/S4E</sup> or PML/RARA<sub>L73E</sub> in Pml<sup>−/−</sup> MEFs (Fig. 6b,c). PML/RARA<sup>F52/S4E</sup> or PML/RARA<sub>L73E</sub> efficiently transformed primary progenitors ex vivo—as assessed by increased clonogenic activity in semi-solid cultures. Yet, the mutants failed to undergo any arsenic-triggered NB re-assembly and terminal differentiation (Fig. 6d and Supplementary Figure 8a,b). Collectively, these data establish the requirement of RING tetramerization in APL development in vivo and arsenic response ex vivo.

**Discussion**

PML RING, B boxes and coiled coil domains cooperate to assemble the insoluble NB scaffolds. We discovered that PML RING, B boxes and coiled coil domains cooperate to assemble the insoluble NB scaffolds. Direct or indirect ensuring efficient UBC9 recruitment. PML sumoylation is unessential for NB-formation but also its sumoylation supports the idea that PML higher order PML RING assembly has endowed PML with the capacity of the

**Methods**

**Protein expression and purification.** The pET32a vector encoding the PML RING<sub>49–119</sub> domain (amino acids 49–104) and a longer RING<sub>119</sub> (amino acids 1–119) were transformed into Escherichia coli BL21 (DE3) (Sangon) for protein production. The design of primer used in this study is shown in Supplementary Table 1 and 2. The recombinant protein containing a N-terminal cleavable (His) tag was induced with 200 μM IPTG (Sangon) and 20 μM ZnCl<sub>2</sub> (Sangon) when the reading of OD<sub>600</sub> reaches 0.8. The cells were grown at 22 °C for 14 h before harvest by centrifugation (4700g, 20 min).

The bacterial cells were resuspended in buffer containing (20 mM Tris, 100 mM NaCl, pH 8.0) and lysed using a cell cracker (INBIO) applying (20 kg cm<sup>−2</sup>) pressure. Cell debris was removed by centrifugation and clean lysate was loaded onto a pre-equilibrated nickel sepharose column (His Trap HP, GE Healthcare). The column was washed with buffer containing (20 mM Tris, 20 mM Imidazole, 500 mM NaCl, pH 8.0) and PML RING was eluted with buffer containing (20 mM Tris, 150 mM Imidazole, 100 mM NaCl, pH 8.0). The TRX-His tag was removed by digestion with Thrombin enzyme at room temperature overnight after the eluate was dialyzed against Thrombin digestion buffer (Sigma). The cleaved TRX-His tag and uncleaved PML RING were removed by recycle over a pre-equilibrated nickel column. PML RING was purified further with an anion exchange sepharose column (Q HP, GE Healthcare) and a hydrophobic interaction sepharose column (Phenyl HP, GE Healthcare). The correct mass of the protein was confirmed by MS analysis, and the purity was checked by SDS-PAGE.

**Crystallization and data collection.** PML RING crystals were grown in 48-well plates using the vapor diffusion technique. Then 0.5 μl PML RING (26 mg ml<sup>−1</sup>) was mixed with 0.5 μl reservoir solution (500 mM Ammonium Sulfate, 1 M Lithium Sulfate and 100 mM Sodium Citrate), and the plates were incubated at 4 °C for ~2 weeks. The crystals were stabilized in a 50:50 mixture of paralin and paratone-N (Hampton Research), and then they were flashed-cooled in liquid nitrogen. Diffraction data were collected in Beamline station BL17U at Shanghai Synchrotron Radiation Facility (SSRF, Shanghai, China).

**Phasing and structure refinement.** The diffraction data were recorded at the wavelength of Zn anomalous dispersion peak (1.284 Å) and subsequently processed, integrated and scaled using MOSFLM/SCALA<sup>25</sup>. The statistics of the data collection are shown in Supplementary Table 1. Single wavelength dispersion (SAD) method implemented in CRANK<sup>25</sup> was used to phase PML RING. Eight Zn<sup>2+</sup> positions were determined by CRUNCH<sup>25</sup> using data between 20 and 1.6 Å. An interpretable map was obtained by solvent flattening using program SOLOMON<sup>25</sup>. The autotracing program ARP/wARP<sup>25</sup> was then used to produce a σ<sub>A</sub>-weighted 2Fo-Fc map for further manual model building. REFMACS<sup>25</sup> was used for structural refinement. Intermittent manual building implemented in COOT<sup>25</sup> was used to correct and improve the initial models produced by ARP/wARP<sup>25</sup>. The B-factors were refined with TLS corrections (4 TLS group, 84 parameters)<sup>25</sup>. The final model of PML RING tetramer contains 187 residues and 224 water molecules.

**Analytical ultracentrifugation and gel filtration analysis.** Sedimentation experiments were conducted using a Beckman XL-I Optima analytical ultracentrifuge equipped with absorbance optics. Sedimentation studies were carried out at 200,000g, at 25 °C overnight. Three-channel with quartz windows were filled with 400 μl of sample (20 mM Tris, 100 mM NaCl, pH 8.0, with/without 1 mM DTT) at the concentration of 1 mg ml<sup>−1</sup>. To investigate how protein concentration might influence PML RING tetramerization, the wild type protein at the higher
**Fig. 5** PML tetramerization controls NB formation and PML sumoylation. 

a | Immortalized Pml−/− MEFs were transduced with MSCV virus expressing PML or its mutants and subsequently transduced or not with CFP-PML. PML NBs were monitored by immuno-fluorescence using anti-HA antibody (red). DAPI is in blue. Scale bar is 5 μm. b | Immortalized Pml−/− MEFs obtained in a were treated with As2O3 (10−6 M, 1 h) and extracts were analyzed by Western blot using anti-HA or anti-CFP. PML and its sumoylated forms are indicated. c | Co-localization of stably expressed HA-PML (red) and UBC9-GFP (green) basal condition (left) or upon 10−6 M As2O3 for 1 h (right). DAPI is in blue. Middle and bottom: Visualization of PML and UBC9 localization with/without arsenic. Scale bars are 5 μm (top) and 0.5 μm (middle and bottom). d | Mammalian two-hybrid: relative luciferase activities (RLU) were used to estimate the interaction between UBC9 and PML/mutants. Statistical significance is indicated. All experiments have been done at least with three independent replicates. Values are means ± S.E. ***p < 0.001 are used to show statistically significant between recombinant derivatives. pACT-PML and pBIND-PML interaction is shown as a positive control.

**Cell culture and treatments.** For expression in MEFs or progenitors, MSCV retroviral constructs were used, HA or CFP tags were in frame with 5′ PML coding sequence. Mutations were generated with the QuikChange II site-directed mutagenesis kit (QIAGEN) on MSCV-HA-His6-PML or pSG5-HA-His6-PML and subcloned in MSCV-HA-His6-PML (named HA-PML) or in MSCV-HA-His6-PML/RARA (HA-PML/RARA). Oligonucleotides used in all the constructs are listed in Supplementary Table 2. Immortalized Pml−/− MEFs, obtained previously by large T expression in immortalized Pml−/− MEF cells, obtained previously by large T expression in CML by large T expression in CML (Promega) using pcDNA3.1(−) vector with T7 promoter according to manufacturer’s standard protocol. The sample was then subjected to gel filtration analysis using Superose 6 and Superose 12 columns (GE) at a flow rate of 0.4 ml min−1. Each fraction was monitored by Western blot using antibody against PML (Abcam).

**Immunofluorescence image acquisition and Western blot.** Cells were fixed with 4% paraformaldehyde. Immunofluorescence assays were performed and analysed by confocal microscopy using the antibodies described below. The slides were examined with a Leica TCS SP8 or Zeiss LSM870 confocal fluorescent microscope. Protein extracts were prepared by lysing cells directly in Laemmli buffer. SUMO conjugates and PML proteins were separated on 4–12% gradient SDS–PAGE (Biorad). Homogeneous chicken polyclonal anti-human PML was previously described45. Mouse monoclonal anti-HA was from Covance, anti-GFP from Roche, rabbit polyclonal anti-CFP and goat polyclonal anti-lamin B antibodies from}

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**Concentration (5 mg ml−1)** was also tested. Absorbance profiles were acquired at a wavelength of 335 nm, chosen according to the protein concentration. Data analysis was carried out using SEDFIT40, which employs the continuous c(s) conformational change model based on the Lamm equation, to determine the sedimentation coefficient distribution.

In order to check RING tetramerization in the context of full-length PML protein, gel filtration analysis was used. Recombinant proteins including PMLABC (i.e., the deletion of residues, 120–360) and mutants were obtained by in vitro translation (Promega) using pcDNA3.1(−) vector with T7 promoter according to manufacturer’s standard protocol. The sample was then subjected to gel filtration analysis using Superose 6 and Superose 12 columns (GE) at a flow rate of 0.4 ml min−1. Each fraction was monitored by Western blot using antibody against PML (Abcam).
Sigma-Aldrich. Anti-mouse cKit (CD117) and anti-mouse Mac1 antibodies were from BD Pharmingen. Alexa 488- or 594-labeled secondary antibodies and HRP-conjugated secondary antibodies from Jackson Laboratories.

For statistics, PML NBs were counted from at least 50 randomly chosen cells. The ratio between sumoylated and unmodified PML was calculated from Vilber Lourmat Fusion camera software. All experiments have been done at least with three independent replicates.

**FRAP analysis.** Fluorescence recovery after photobleaching (FRAP) was performed on Zeiss LSM510 confocal microscope equipped with a heated chamber. MEF cells expressing HA-PMLwt or HA-PMLF52/54A together with UBC9-GFP were seeded in glass-bottomed dish in HEPES-buffered DMEM media with 10% FBS and the dish was mounted on the stage in the confocal chamber pre-heated to 37 °C. The cells were observed under a 63× oil lens. The “region of interest” (ROI) containing only one PML NB was selected, and UBC9-GFP was bleached with a 488 nm argon laser at 100% intensity for 6 times. The means of relative intensity of UBC9-GFP fluorescence during fluorescent recovery were quantified from three independent experiments.

**Transgenic mice.** PML/RARA and PML/RARA<sub>L73E</sub> were expressed using human MR8 promoter<sup>42</sup>. Mice were housed in specific pathogen-free conditions and all animal experiments were approved by the Animal Care and Use Committee at Experimental Animal Center in Shanghai Jiao Tong University School of Medicine. To define whether the mice have developed APL, hematological disorders such as splenomegaly, MGG staining and c-Kit, GR1 and Mac-1 flow cytometry analysis. Scale bar is 5 μm. e Sumoylation of HA-PML/RARA after 1 h of As<sub>2</sub>O<sub>3</sub> exposure was monitored by Western blot using anti-RARA antibody<sup>47</sup>. d MGG staining of mouse hematopoietic progenitors transformed by PML/RARA and the indicated mutants after arsenic treatment (10<sup>−7</sup>M, 7 days). e Proposed model for NB assembly and PML sumoylation integrating the PML RING tetramer formation.

**Fig. 6** PML RING tetramerization is important for APL development and arsenic targeting of PML/RARA-transformed cells. a Survival data of MR8-PML/RARA or -PML/RARA<sub>L73E</sub> transgenic mice<sup>42</sup>. b Pml<sup><s>-/-</s></sup> MEFs expressing HA-PML/RARA, HA-PML/RARA<sub>F52/54A</sub> or HA-PML/RARA<sub>L73E</sub> were treated with As<sub>2</sub>O<sub>3</sub> (10<sup>−6</sup>M) prior to immunofluorescence analysis. Scale bar is 5 μm. c Survival data of MRP8-PML/RARA<sub>L73E</sub> and mutants were subjected to SEC-MALS analysis (S100 column, GE Healthcare). The elution peaks, as monitored by UV absorption at 280 nm, were pooled separately and chosen for size exclusion chromatography-multi-angle light scattering (SEC-MALS) characterization, respectively. In brief, the purified protein samples were concentrated and analyzed using a WTC-0155 size exclusion column (Wyatt Technology) which was connected to a 1260 infinity liquid chromatography system (Agilent Technology) equipped with inline DAWN HELOS-II MALS and Optilab rEX differential refractive index detectors (Wyatt Technology). For each sample, a 40 μl injection volume and 0.5 ml min<sup>−1</sup> flow rate were applied. Data were recorded and processed using ASTRA VI software (Wyatt Technology).

**SEC-MALS analysis.** The purified PML RING<sub>2-119</sub> and mutants were subjected to gel filtration analysis (5100 column, GE Healthcare). The elution peaks, as monitored by UV absorption at 280 nm, were pooled separately and chosen for size exclusion chromatography-multi-angle light scattering (SEC-MALS) characterization, respectively. In brief, the purified protein samples were concentrated and analyzed using a WTC-0155 size exclusion column (Wyatt Technology) which was connected to a 1260 infinity liquid chromatography system (Agilent Technology) equipped with inline DAWN HELOS-II MALS and Optilab rEX differential refractive index detectors (Wyatt Technology). For each sample, a 40 μl injection volume and 0.5 ml min<sup>−1</sup> flow rate were applied. Data were recorded and processed using ASTRA VI software (Wyatt Technology).

**Small angle X-ray scattering.** The recombinant PML RING<sub>49-104</sub> was purified and concentrated to 1, 3 and 5 mg ml<sup>−1</sup> in 20 mM Tris pH 8.0, 100 mM NaCl, with/without 1 mM DTT, respectively. The X-ray scattering experiment was carried out at Beamline station BL19U2 (National Facility for Protein Science Shanghai, NCPSS, China) and scattered X-ray intensities were collected by using a Pilatus 1 M detector (DECTRIS Ltd). The measurements were carried out with 0.5 ms per frame and repeat for 20 times to avoid possible sample radiation damage. The collected data were processed with ATSAS software package<sup>43</sup>. The details of SAXS data collection and processing are shown in Supplementary Table 3. The guiner region of experimental group are linear, indicating the measured sample is homogeneous in solution. Crystal data collection and processing are shown in Supplementary Table 3. The guiner region of experimental group are linear, indicating the measured sample is homogeneous in solution. Crystal data collection and processing are shown in Supplementary Table 3. The guiner region of experimental group are linear, indicating the measured sample is homogeneous in solution. Crystal data collection and processing are shown in Supplementary Table 3. The guiner region of experimental group are linear, indicating the measured sample is homogeneous in solution. Crystal data collection and processing are shown in Supplementary Table 3.
Mammalian two-hybrid assay. Mammalian two-hybrid assay was performed using the CheckMate™ Mammalian Two-Hybrid System (Promega) in 293 T and CHO cells. The cDNA of UBC9 and full length WT PML or mutants were inserted into pBIND and pACT vectors, respectively. 293T cells were then transfected with plasmids pG5/luc, pBIND-UBC9 and pACT-PML/mutant (mixed at a molar ratio of 1:1) using liposome Lipo2000 transfection method (Life Technology). In the complementary set of experiments, UBC9 was cloned into pACT and the extended RING domain into pBIND. The resulting plasmids were transfected in CHO cells. The cDNA of UBC9 and full length WT PML or mutants were inserted into pBIND and pACT vectors, respectively. Twenty-four hours after transfection, luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega).

Limited proteolysis. 10 μg purified PML RING1–193 was subjected to chymotrypsin (Sigma) digestion at 25 °C for 20 min. The reaction mixture contained 10 μg RING1–193, a series dilutions from 10 μg chymotrypsin, 100 mM Tris (pH 8.0), 10 mM CaCl2, and 1 mM DTT. The reaction was terminated by boiling with SDS loading buffer. The sample was analyzed by SDS–PAGE followed by silver staining. Mass spectrometry analysis was conducted with 5800 MALDI-TOF/TOF (AB Sciei). The limited digestion product was subjected to the PVDVF (GE Healthcare) membrane transferring followed by Ponceau S staining to indicate the target protein band. Cropped PVDVF membrane containing the target protein was placed into the reactor of the PPSS-33A (SHIMADZU) automatic protein sequencer, followed by a standard analysis procedure.

Table 1 Data collection and structure refinement statistics of PML-RING

| Data collection | | |
|---|---|---|
| Space group | P2_12_12_1 | |
| Unit cell dimension (Å) | a 38.5 | b 84.7 | c 86.1 |
| Molecule per ASU | 4 | |
| Derivative | Native | |
| Source/Station | BL17U | |
| Wavelength (Å) | 1.2824, Zn Peak | |
| Resolution range (Å) | 60.4 – 1.60 | |
| Observations (I/σ(I) > 0) | 467248 | |
| Unique reflections (I/σ(I) > 0) | 37754 | |
| High resolution shell (Å) | 1.69-1.60 | |
| Rmerge (%) | 14.9 (133.6) | |
| Completeness (%) | 99.4 (99.9) | |
| Redundancy | 2.4 (11.5) | |
| CC1/2 | 0.996 (0.848) | |
| Structure refinement | Resolution range (Å) | 60.4 – 1.60 | |
| R-factor (%) | 20.3 | |
| R-free (%) | 35.0 | |
| Rmerge (%) | 21.7 | |
| Rfree (high resolution shell) | 36.6 | |
| Total number of non-hydrogen atoms | 1403 Protein atoms | 224 Water molecules | 8 Zn ions | 8 R.m.s. deviations | Bond length (Å) 0.005 | Bond angle (°) 0.954 | Main chain B-factors (Å²) 1.799 | Side chain B-factors (Å²) 5.871 | Wilson B-factor (Å²) 22.1 | Average B-factor (Å²) 43.8 Protein atoms | 49.5 Solvent atoms | 38.3 Zn ions | 99.4 Ramachandran statistics (%) | Most favored region | Allowed regions 0.6 |

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Data availability. Coordinates and structure factors have been deposited in the Protein Data Bank under accession code 5YUF. Other data are available from the corresponding authors upon request.
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Additional information

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