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# TRAIP regulates Histone H2B monoubiquitination in DNA damage response pathways

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**Abstract.** Histone H2B monoubiquitination has been shown to play critical roles in diverse cellular processes including DNA damage response. Although recent data indicate that H2B monoubiquitination is strongly connected with tumor progression and regulation, the implications of this modification in lung adenocarcinoma are relatively unknown. In the present study, we demonstrated the clinical implication of H2B monoubiquitination and the potential role of tumor necrosis factor receptor-associated factor-interacting protein (TRAIP) in regulating its modification in lung adenocarcinoma. Immunohistochemical analysis showed that H2B monoubiquitination was significantly downregulated in 68 human lung adenocarcinoma patient samples compared to their normal adjacent tissues. Depletion of TRAIP by specific siRNA treatment markedly decreased ionizing radiation (IR)-induced H2B monoubiquitination. In addition, deletion mutants without RING domain or C-terminus of TRAIP diminished the ability to induce H2B monoubiquitination at lysine 120. Notably, the nuclear expression of TRAIP was positively related with H2B monoubiquitination levels in patients with lung adenocarcinoma. Furthermore, statistical analysis indicated that low levels of both TRAIP and H2B monoubiquitination, not each alone, in patients with lung adenocarcinoma were strongly correlated with poor survival. Taken together, these results suggest that TRAIP is a novel regulator of H2B monoubiquitination in DNA damage response and cancer development in lung adenocarcinoma.

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## Introduction

The DNA damage response causes numerous cascades of post-translational modifications including ubiquitination, SUMOylation, acetylation and phosphorylation that coordinate the cell cycle, apoptosis and the DNA damage repair pathway (1,2). Histone modification is one of the important events in the DNA damage response pathway and regulates recruitment of other proteins to DNA damage sites, chromatin remodeling and the enzymatic signaling pathway during DNA damage (3,4).

Histone H2B can be post-translationally modified at its N-terminal and C-terminal tail like other histone proteins. Monoubiquitination at the Lys-120 (K120) residue of H2B is associated with gene transcription, improper development, tumorigenesis and defects in the differentiation of stem cells (5,6). In humans, K120-monoubiquitination of H2B is known to be catalyzed by Ring finger protein (RNF)20 and RNF40 E3 ligases in conjunction with ubiquitin-conjugating enzyme UbcH6 (7,8).

K120-monoubiquitination of H2B also regulates chromatin compaction and RNF8/RNF168-dependent transcriptional silencing triggered near double-strand break sites in mammals (9-11). Accordingly, H2B monoubiquitination is linked to positive regulation of transcription. UbcH6 and RNF20/40, E2 conjugating enzyme and E3 ligase of H2B respectively, are recruited to transcriptionally active sites. Monoubiquitination of H2B also facilitates histone chaperone FACT and promotes transcription elongation (12). In addition, downregulation of H2B ubiquitination is also related with

cancer development. USP22 which is a deubiquitinase of monoubiquityl H2B is related with aggressive growth, metastasis, and therapy resistance, whereas RNF20, ubiquitinase of H2B was reported to be a putative tumor-suppressor (13,14). RNF20 and RNF40 are recruited to DNA double-strand break sites and induce K120-monoubiquitination of H2B to regulates the DNA damage response pathway in an ATM-dependent manner (15).

TRAIP (TRAF-interacting protein) has been identified as a TNF receptor-associated factor 1 (TRAF1) and TNF receptor-associated factor 2 (TRAF2)-binding partner which contains N-terminal really interesting new gene (RING) domain motif (16). Like other TNF receptor-associated factor proteins, TRAF1 and TRAF2 contribute to tumor necrosis factor (TNF) receptor-induced signal transduction pathways including the NF-κB signaling pathway and TRAIP inhibits NF-κB activation through interaction with TRAF proteins (16,17). TRAIP is known to inhibit NF-κB activation by interacting with CYLD or Syk independently of its E3 ubiquitin ligase activity (18-20). Most RING containing proteins act as ubiquitin ligase or mediate ubiquitin transfer reaction and TRAIP also has an E3 ubiquitin ligase function on self-ubiquitination (18,21). Recent studies have also noted that TRAIP is a regulator of mitotic progression and DNA damage response (22,23). In particularly, we and other authors have demonstrated that TRAIP promotes homologous recombination and protects genomic stability after replication stress, and deficiency of normal TRAIP is related to genomic instability-related diseases such as primordial dwarfism and lung cancer (23-26). One study demonstrated that the E3 ubiquitin ligase activity of TRAIP is essential for its role in the DNA damage response by showing the promotion of RPA loading and phosphorylation after replication stress (26). We also reported that RNF20 and RNF40 interact with TRAIP and recruit TRAIP to DNA damage sites to regulate the DNA damage response pathway (25).

Since the RNF20/40 complex controls the DNA damage response pathway by inducing H2B monoubiquitination and recruiting TRAIP to the DNA damage sites, we aimed to ascertain whether TRAIP participates in the regulation of H2B monoubiquitination in response to DNA damage.

In the present study, we confirmed that TRAIP controls H2B monoubiquitination through the RING domain and C-terminus. In addition, we further confirmed that the nuclear expression of TRAIP is correlated with H2B monoubiquitination. Furthermore, the expression levels of these two proteins are related to patient survival. Taken together, these results suggest that TRAIP is a novel regulator of H2B monoubiquitination in DNA damage response and cancer development in lung adenocarcinoma.

## Materials and methods

*Plasmids and antibodies.* A Myc-tagged TRAIP expression plasmid was previously described (25). To remove the off-targeting effects of RNAi-mediated knockdown of TRAIP, we constructed an siRNA-resistant TRAIP wild-type expression vector (TRAIP-R) by site-directed mutagenesis using the Myc-tagged TRAIP expression plasmid as shown in Fig. 3A. TRAIP-R-D1 and TRAIP-R-D2 expression plasmids (Fig. 3B) were generated by mutagenesis using the TRAIP-R vector. Rabbit anti-human TRAIP polyclonal antibody was raised by immunizing rabbits with the GST-TRAIP fusion protein as previously described (25). Antibodies against Myc (cat. no. 11814150001; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), histone H3 (cat. no. sc-10809; Santa Cruz Biotechnology, Dallas, TX, USA) and β-actin (cat. no. A5316; Sigma-Aldrich; Merck KGaA) were used. Anti-Histone H2B (cat. no. 07-371) and anti-ubiquityl Histone H2B (cat. no. 05-1312) antibodies were purchased from Sigma-Aldrich; Merck KGaA). The dilution ratio of each antibody for western blotting was as follows: Anti-TRAIP, 1:400; anti-Myc, 1:2,000; anti-H3, 1:1,000: Anti-Histone H2B, 1:2,000; anti-ubiquityl Histone H2B 1:2,000; and anti-β-actin, 1:5,000. HRP-conjugated secondary antibodies specific to rabbit (cat. no. A0545; Sigma-Aldrich; Merck KGaA) or mouse (cat. no. A9917; Sigma-Aldrich; Merck KGaA) IgG were used at a dilution of 1:2,000.

*Cell culture, siRNAs, transfection and irradiation.* HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM; WelGENE Inc., Gyeongsangbuk-do, Korea), supplemented with 10% fetal bovine serum (FBS; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), 100 U/ml penicillin and 100 $\mu$g/ml streptomycin (Invitrogen; Thermo Fisher Scientific, Inc.). The cells were maintained at 37˚C in a humidified atmosphere containing 5% $CO_2$. The control siRNA was previously described (27,28). The TRAIP siRNA sequences were as follows: TRAIP#1, 5'-GCAGCAGGAUGAGACCAAAUU-3' and TRAIP#2, 5'-GCAAGUUGCAGACAGUCUAUU-3'. All siRNAs were purchased from Noble Bio (Hwaseong, Korea). Cells were transfected with 50 nM siRNAs using DharmaFECT 1 (Dharmacon, Inc., Lafayette, CO, USA) and DNA transfection was performed using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) as per the instructions of the manufacturer. Twenty-four hours after plasmid transfection or 48 h after siRNA transfection, transfected cells were irradiated at the indicated doses by a gamma-irradiator (IBL437C; CIS Bio Inc., Saclay, France) and $^{137}$Cs was used for ionized radiation source.

*Mutagenesis and reconstruction experiments.* Myc-tagged siRNA-resistant TRAIP was generated by silent mutagenesis of siRNA-targeted sequence using a Myc-tagged TRAIP expression plasmid. Myc-tagged siRNA resistant D1 and D2 of TRAIP were generated by further mutagenesis using a Myc-tagged siRNA-resistant TRAIP plasmid. HeLa cells were transfected with siRNAs using DharmaFECT 1 (Dharmacon, Inc.) as per the instructions of the manufacturer. Twenty-four hours after siRNA transfection, siRNA-resistant plasmid DNA transfection was performed using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) as per the instructions of the manufacturer. Twenty-four hours after DNA transfection, cells were irradiated. Irradiated cells were harvested and analyzed by chromatin fraction and western blotting after indicated times since cells were irradiated.

*Western blotting.* HeLa cells were transfected with siRNA and expression plasmids as indicated in each figure legend.

Forty-eight hours after siRNA or 24 h after DNA transfection, cells were lysed in NETN buffer [0.5% Nonidet P-40, 20 mM Tris (pH 8.0), 50 mM NaCl, 50 mM NaF, 100 $\mu$M $Na_3VO_4$, 1 mM dithiothreitol and 50 $\mu$g/ml phenylmethylsulfonyl fluoride] for 20 min on ice. Crude lysates were cleared by centrifugation at 12,300 x g for 15 min at 4˚C. A total of 15 $\mu$g samples of supernatants or chromatin fraction were subjected to each lane of 12% SDS-PAGE gels. After electrophoresis, the separated proteins were transferred to a polyvinylidene difluoride (PVDF) membrane and blocked using 1X Tris-buffered saline with 0.5% Tween-20 (TBST) with 1% w/v non-fat dry milk for 30 min at room temperature. The membranes were incubated with primary antibodies in blocking buffer for 16 h at 4˚C. After washing with 1X TBST, secondary antibodies were applied with blocking buffer and further incubated for 1 h at room temperature. Visualization reagent (AbSignal, ABC-3001) was purchased from AbClon Inc. (Seoul, Korea). Band densities were quantified using Image Lab (version 5.2; Bio-Rad Laboratories, Hercules, CA, USA).

*Chromatin fraction*. Cells were collected and washed in phosphate-buffered saline (PBS). The collected cells were lysed in NETN buffer [0.5% Nonidet P-40, 20 mM Tris (pH 8.0), 50 mM NaCl, 50 mM NaF, 100 $\mu$M $Na_3VO_4$, 1 mM dithiothreitol and 50 $\mu$g/ml phenylmethylsulfonyl fluoride] at 4˚C for 30 min. Crude lysates were cleared by centrifugation at 16,000 x g at 4˚C for 10 min and the pellet was suspended in HCl (0.2 M) for 1 h. The resuspended mixture was centrifuged at 16,000 x g at 4˚C for 10 min, and then the lysate which is the chromatin fraction was neutralized with Tris-HCl (1 M at pH 8.0) for western blotting. We used histone 3 (H3) as a control for the chromatin fraction.

*Tissue microarrays and immunohistochemistry*. A tissue microarray containing 75 cases of human lung adenocarcinoma tissues and each matched normal adjacent tissue (HLug-Ade150Sur-01) with no information regarding the treatment and therapy received by patients was obtained from US Biomax, Inc. (Rockville, MD, USA). Immunohistochemical staining was performed as previously described (21) with a rabbit polyclonal anti-TRAIP antibody (PA5-27699; Thermo Fisher Scientific, Inc.) and a rabbit monoclonal anti-ubiquityl-H2B antibody (cat. no. 5546S; Cell Signaling Technology, Inc., Danvers, MA, USA) at a concentration of 1:200 and 1:500, respectively. The staining intensity was assigned an arbitrary value, on a 4-scale (intensity score) as follows: Non-stained (0), weak (1), moderate (2), and strong (3). The H-score was calculated by multiplying the intensity score and the fraction score (percentage of counted samples at each scale), producing the range 0-300, and repeated on three different areas.

*Statistical analysis*. Statistical analysis was performed using GraphPad Prism 5 program (GraphPad Software, Inc., San Diego, CA, USA). Values were generally represented as mean of H-scores ± standard error of the mean (SEM). A Box-and-Whisker plot was also used to compare the distribution of each sample. Comparison of TRAIP expression in the nucleus of tumor and adjacent non-tumor groups was analyzed using t-test. Pearson correlation coefficient (r) analysis was used to compare the expression of TRAIP and H2B in the nucleus of tumor cells. The method of Kaplan-Meier and the log-rank test were used for comparing overall survival between groups. A two-sided P-value of <0.05 was considered to indicate a statistically significant result.

## Results

*H2B monoubiquitination is downregulated in human lung cancer patient samples*. To determine whether H2B monoubiquitination is related with lung cancer, we determined the expression levels of H2B monoubiquitination in human lung cancer patient tissues and normal adjacent tissues (≥2 cm away from every cancer tissue) using a tissue microarray containing 75 cases of each. We found that the expression levels of H2B monoubiquitination in tumors were much lower than that noted in the non-tumor tissues (Fig. 1A). H-scoring in every tissue confirmed that H2B monoubiquitination was greatly decreased in human lung cancer patients (median H-score, 72.3) compared with that in each matched normal adjacent tissue (median H-score, 123.3) (Fig. 1B). The comparison of the results indicated that H2B monoubiquitination in 60.3% of the cancer tissues was at least 1.5-fold lower than that in each corresponding matched normal tissue.

*TRAIP depletion decreases ionizing radiation (IR)-induced H2B monoubiquitination*. The structure of the RING finger domain of TRAIP is conserved widely across mammals (Fig. 2A). This conservation implies the importance of RING domain for the function of TRAIP, and RING finger domain usually interacts with ubiquitination enzyme and has E3 ligase activity (29). In addition, we confirmed that the RNF20-RNF40 complex, which is an E3 ubiquitin ligase for H2B monoubiquitination at the sites of DNA damage, regulates translocation of TRAIP to DNA damage site leading to the regulation of the DNA damage response pathway and homologous repair which is induced by H2B monoubiquitination (25). Based on these results, we hypothesized that TRAIP may participate in monoubiquitination of H2B during the DNA damage response. To verify this hypothesis, we depleted TRAIP with specific siRNAs and the cells were exposed to IR. IR increased H2B monoubiquitination without any effect on the expression of H2B in the chromatin fraction, but the IR-mediated H2B monoubiquitination was decreased in the TRAIP-knockdown cells (Fig. 2B). We also confirmed that IR induced H2B monoubiquitination at the K120 residue using the anti-ubiquityl H2B (Lys-120) antibody and that knockdown of TRAIP diminished the K120-H2B monoubiquitination (Fig. 2C). These results indicated that TRAIP is necessary for the DNA damage-mediated K120-monoubiquitination of H2B.

*RING domain and C-terminal of TRAIP is crucial for IR-mediated H2B monoubiquitination*. Since previous reports noted that the RING domain of TRAIP has the E3 ubiquitin ligase activity and C-terminal region of TRAIP is involved in translocating its DNA damage sites (18,25),

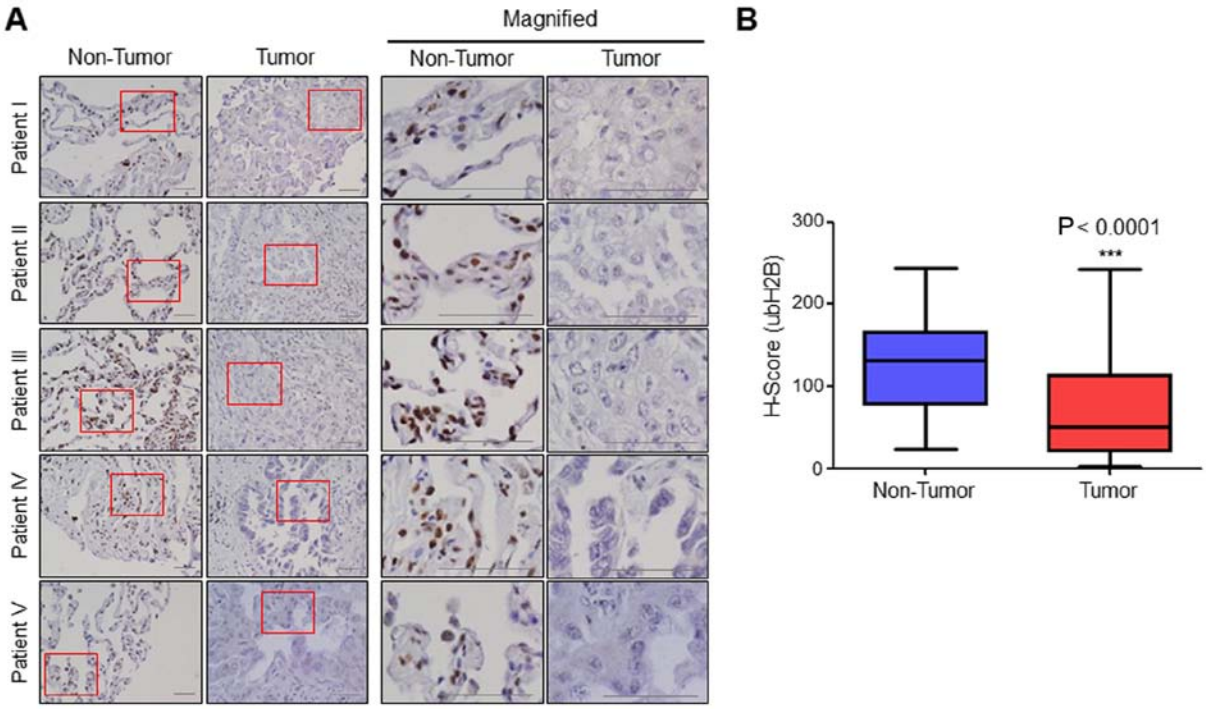


Figure 1. Monoubiquitination of H2B is significantly reduced in lung cancer patient tissues. (A) Immunohistochemical staining of ubH2B in human lung cancer patient tissues (Tumor) and matched normal adjacent tissues (Non-Tumor). Red squares indicate the magnified region shown in the right panel. Scale bar, 50 $\mu$m. (B) Box-and-Whisker plots of ubH2B H-scores in lung cancer (Tumor) and normal adjacent tissues (Non-Tumor). $^{***}$P<0.0001 (n=68 for each tumor and normal adjacent sample). H2B, histone 2B; ubH2B, H2B monoubiquitination.

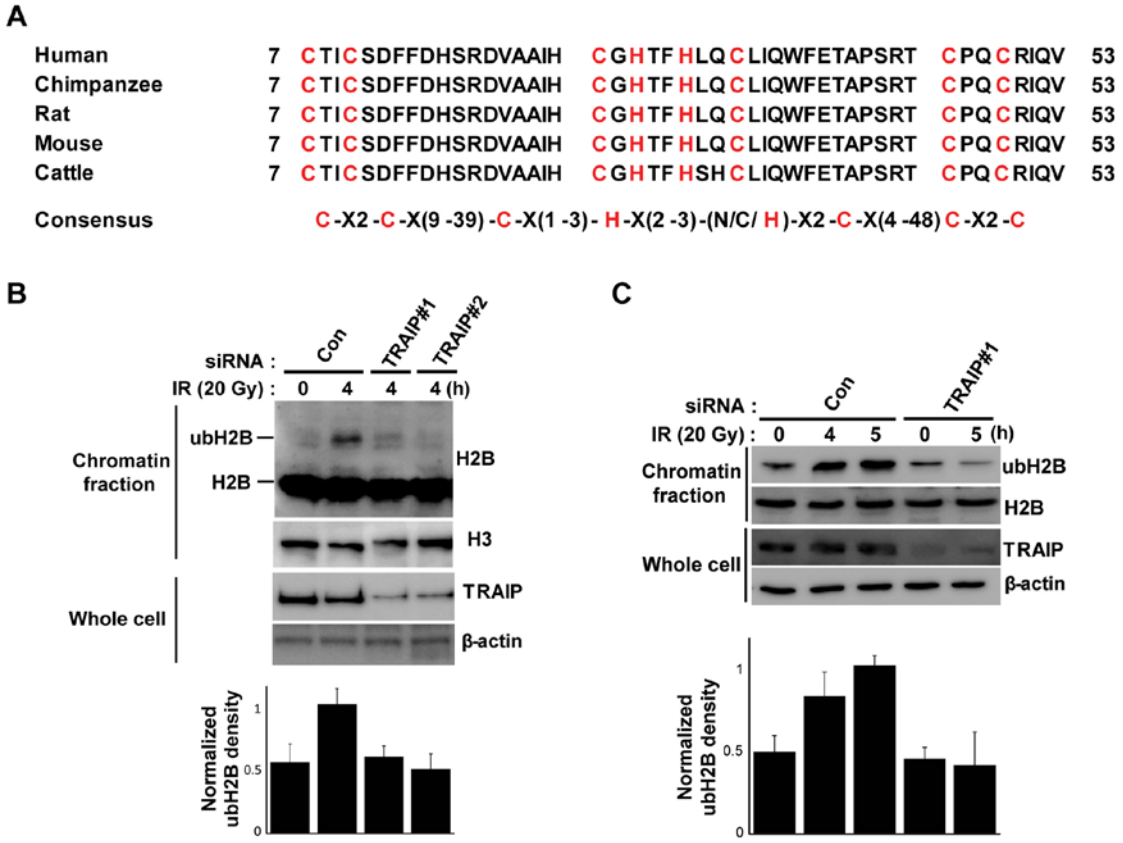


Figure 2. TRAIP is required for IR-mediated H2B monoubiquitination. (A) RING domain of TRAIP is conserved in the human, chimpanzee, rat, mouse and cattle. (B and C) HeLa cells ($5x10^6$) in a 60-mm-diameter plate were transfected with control or TRAIP siRNA for 48 h and then the transfected cells were treated with 20 Gy of IR. Chromatin fraction or whole cell lysates of the treated cells were prepared for western blotting using indicated antibodies. H2B expression was used as a control to normalize the expression of ubH2B in the representative diagram and the data are expressed as the mean ± standard deviation (n=3). H2B, histone 2B; ubH2B, histone 2B lysine 120 monoubiquitinated; TRAIP, TRAF-interacting protein; IR, ionizing radiation; RING, really interesting new gene.

we next examined which domain of TRAIP is involved in the IR-mediated H2B monoubiquitination. First, to remove the off-targeting effects of RNAi-mediated knockdown of TRAIP, we constructed the siRNA-resistant TRAIP wild-type expression vector (TRAIP-R) with silent mutation and checked the siRNA-resistant feature. HeLa cells were transfected with non-targeting (control) or TRAIP-targeting siRNA and 24 after siRNA transfection, the cells were secondly transfected with a plasmid carrying wild-type TRAIP (WT) or siRNA-resistant wild-type TRAIP (TRAIP-R) as indicated (Fig. 3A). The reconstitution using the TRAIP-R in TRAIP-depleted cells successfully rescued the IR-mediated H2B monoubiquitination (Fig. 3C). These results confirmed again that TRAIP is a key regulator for H2B monoubiquitination during the DNA damage response. We then generated TRAIP-deletion mutants without the RING domain (R-D1) or C-terminus (R-D2) using the TRAIP-R as the template (Fig. 3B). As shown in Fig. 3C, both deletion mutants, R-D1 and R-D2 failed to rescue the IR-mediated H2B monoubiquitination, suggesting that both RING domain (E3 ubiquitin ligase activity) and C-terminus (TRAIP recruitment to DNA lesion) are essential for inducing H2B monoubiquitination.

*Expression of nuclear TRAIP positively correlates with H2B monoubiquitination and lower expression of both is associated with poor prognosis in patients with lung cancer.* We then investigated a possible correlation between the expression of TRAIP and H2B monoubiquitination in human lung cancer patient tissues to evaluate the clinical importance of the relationship. For this purpose, we examined the immunostaining patterns of nuclear TRAIP and H2B monoubiquitination in the same region of every tissue using consecutive sections. As we previously reported (25), nuclear TRAIP expression in lung cancer patient tissues was lower than that in each matched normal adjacent tissue. Intriguingly, we also observed a positive correlation between the nuclear expression of TRAIP and H2B monoubiquitination in the tumor tissues (Fig. 4A). Decreased H2B monoubiquitination was found in the patient tissues with a lower nuclear expression of TRAIP while increased H2B monoubiquitination in the tumors with a higher nuclear expression of TRAIP. A scatter plot and Pearson correlation coefficient analysis revealed a significant positive correlation (r=0.4052) between the nuclear expression of TRAIP and H2B monoubiquitination (Fig. 4B). In addition, comparison among three patient groups divided by the sum of H-scores for nuclear TRAIP and H2B monoubiquitination indicated that patient survival of the high expression group was considerably longer than the other two groups, middle (P=0.0059) and low group (P=0.026) (Fig. 4C) even though neither nuclear TRAIP nor H2B monoubiquitination alone showed any meaningful correlation with patient survival (Fig. 4D and E), suggesting that decreased expression of both nuclear TRAIP and H2B monoubiquitination is significantly associated with shorter survival of lung cancer patients. Together these results indicated that the expression of nuclear TRAIP positively correlates with H2B monoubiquitination and a decrease in both nuclear TRAIP and H2B monoubiquitination is significantly associated with a poor prognosis in patients with lung cancer.

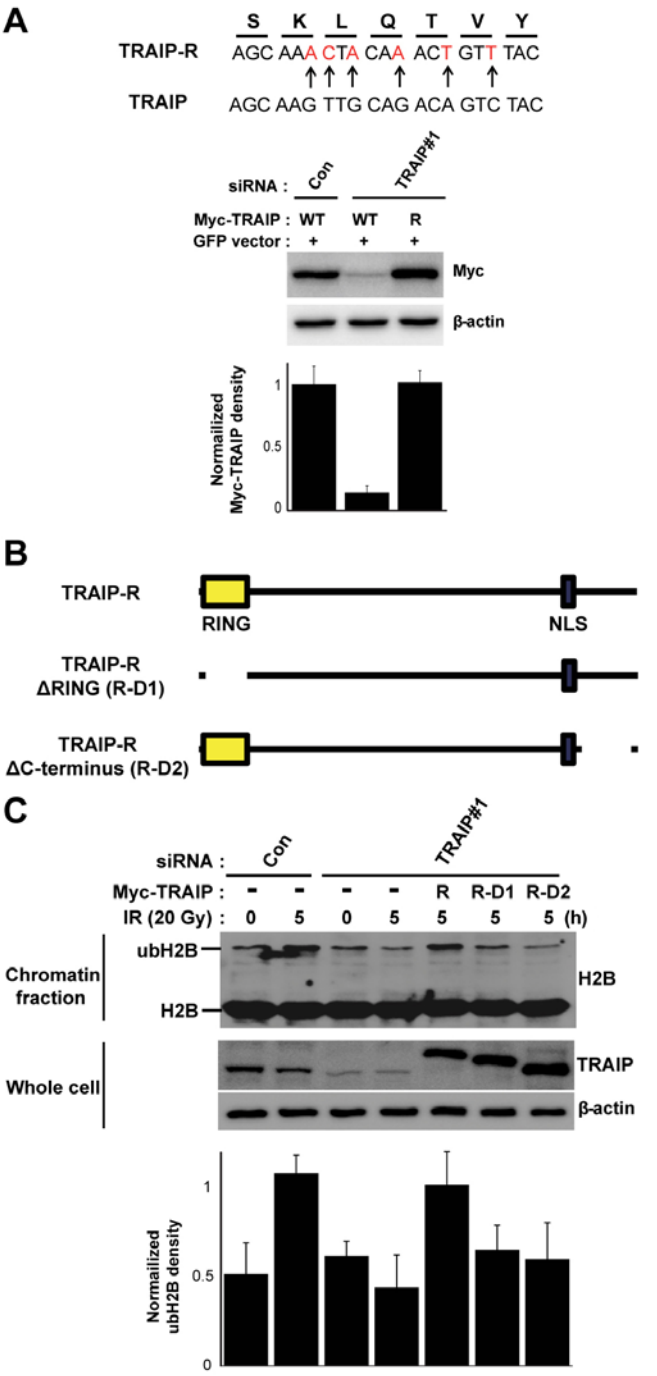


Figure 3. RING domain and C-terminal region of TRAIP are instrumental in IR-mediated H2B monoubiquitination. (A) The upper panel shows mutated sequences of the siRNA-resistant TRAIP wild-type expression plasmid containing the silent mutation (TRAIP-R). In the lower panel, $5x10^6$ of HeLa cells in a 60-mm-diameter plate were transfected with the siRNA and plasmid as indicated for 48 h and then whole cell lysates were prepared for western blotting to confirm that the TRAIP-R construct is resistant to the TRAIP siRNA treatment. β-actin expression was used for as a control to normalize the Myc-TRAIP wild-type and R expression in the representative diagram and the data are expressed as the mean ± standard deviation (n=3). (B) Structure of TRAIP-R expressing wild-type TRAIP protein and internal deletion mutants of TRAIP. (C) HeLa cells ($5x10^6$) in a 60-mm-diameter plate were transfected as indicated for 48 h. The transfected cells were exposed to 20 Gy of IR for 5 h. Cells then were harvested and the chromatin fraction and whole cell lysate of the cells were subjected to western blot analysis using indicated antibodies. H2B expression was used as a control to normalize the expression of ubH2B in the representative diagram and the data are expressed as the mean ± standard deviation (n=3). RING, really interesting new gene finger domain; TRAIP, TRAF-interacting protein; NLS, nuclear localization signal; ubH2B, H2B monoubiquitination; H2B, histone 2B.

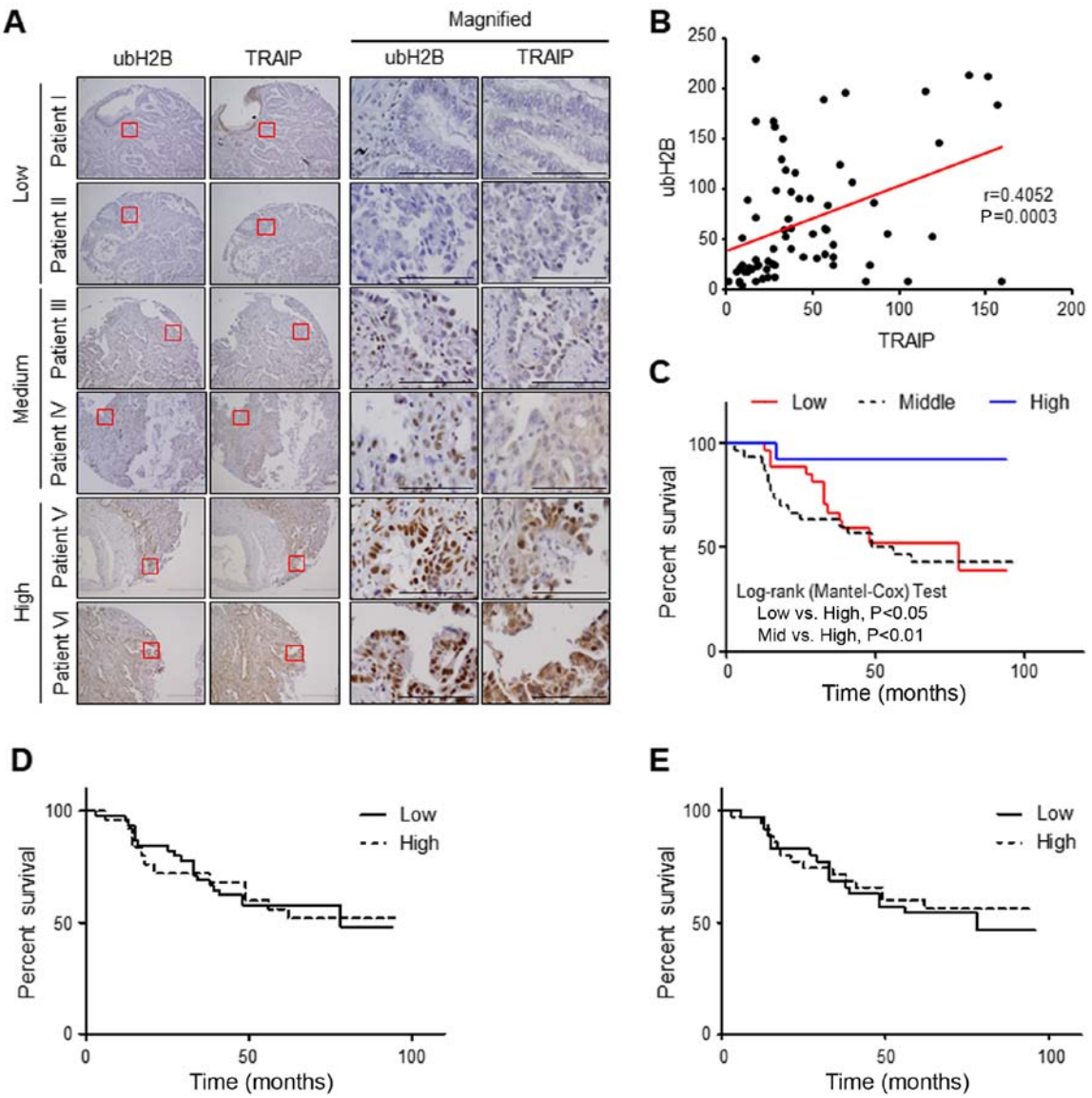


Figure 4. Correlation between nuclear TRAIP expression and ubH2B in lung cancer patient samples. (A) Immunohistochemical staining of TRAIP and ubH2B in human lung cancer patient tissues. Red squares indicate the magnified region shown in the right panel. Scale bar, 200 $\mu$m and scale bar in the magnified figure, 50 $\mu$m. (B) Pearson correlation coefficient (r) between the nuclear expression of TRAIP and ubH2B. The graph presents the scatter plot of the H-score of nuclear TRAIP (x-axis) and ubH2B (y-axis). P=0.0003 (n=70). (C) Patients in the high expression group of both analytical factors show longer overall survival than in the middle (P=0.0059) or low group (P=0.0226). No significant difference between low and middle groups (P=0.53) was noted. High expression group (blue solid line, n=13) with sum H-score (sum of H-scores for TRAIP and ubH2B) >180, middle group (black dot line, n=30) with 80< sum-score <180, and low group (red solid line, n=27) with sumH-scoring <80. (D) No significant difference between low and high groups (P=0.9974). High group (dotted line, n=35) with H-score for TRAIP expression >50, low group (solid line, n=35) with <50. (E) No significant difference between low and high groups (P=0.8806). High group (dotted line, n=25) with H-score for ubH2B expression >50, low group (solid line, n=45) with <50. TRAIP, TRAF-interacting protein; ubH2B, H2B monoubiquitination.

## Discussion

Lung cancer is the most common cancer in regards to incidence accounting for 13% of all newly diagnosed cancer cases, and lung cancer-related mortality accounts for 18% of the total cancer-related deaths globally (30). Lung cancer is caused by diverse factors, such as smoking, air pollution, diet and genetic mutations (31). Non-small cell lung cancer (NSCLC) is the main lung cancer subtype with 85% of patients and has two major subtypes, lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) (32). Although many different types of interventions including targeted therapy have been developed to deal with cancer to date, most patients with lung cancer are treated with cytotoxic chemotherapeutic drugs such as cisplatin, carboplatin, docetaxel, and gemcitabine, which mainly induce DNA damage (33).

A primary hallmark of cancer is genomic instability that is induced by the accumulation of DNA damage. Cellular DNA damage caused by various carcinogens including cytotoxic drugs stimulates many types of enzymes and signaling molecules leading to cell cycle arrest, regulation of DNA replication and the repair of DNA damage, which is called DNA damage response (DDR) (34). Many molecules participating at DDR have been classified by their roles as DNA damage sensors, recruitment mediators, transducers, and effectors to initiate signaling pathways to impact various cellular responses (35).

The combination of radiotherapy and DNA damage-inducing chemotherapy is the classic and effective strategy for overall tumor treatment, even though serious side effects occur. To diminish unwanted adverse reactions, novel drugs targeting the molecules involved in DDR has been recently developed; for example, olaparib (Lynparza®), the poly(ADP-ribose) polymerase (PARP) inhibitor (36).

Histone modifications including methylation, ubiquitination, phosphorylation and acetylation have been considered the essential events for DDR by forming recruitment platforms for downstream effectors and guiding the activity of chromatin remodelers (4). In particularly, H2B monoubiquitination is an important histone modification in transcriptional regulation and chromatin organization even in the absence of DNA damage (37), and also has a pivotal role in the recognition of DNA damage sites as a regulator of damage checkpoint activation and timely initiation of repair (38,39). H2B monoubiquitination has been known to be generated by RNF20/RNF40, RING finger proteins, with E3 ubiquitin ligase activity (40-43). TRAIP also exists in the nucleus and the cytoplasm, but DNA damage increases its nuclear translocation, especially to damaged sites (23,25,44). We also reported that TRAIP induces DDR and repair and that RNF20/RNF40 regulates translocation of TRAIP to DNA double-strand breaks (25). In the present study, we demonstrated that TRAIP is a novel regulator of H2B monoubiquitination, further supporting that TRAIP is an essential component in DDR. Furthermore, in the present study, a positive correlation was confirmed between nuclear expression of TRAIP and H2B monoubiquitination in lung cancer patient samples and that decreased expression of both nuclear TRAIP and H2B monoubiquitination was closely related with shorter survival of lung cancer patients (Fig. 4). As discussed above, genomic instability is one of primary hallmarks of cancer development and progression, and is also increased by therapeutic treatment of cancer patients by chemotherapy or radiotherapy. These results strongly suggest that dysregulation of TRAIP and H2B monoubiquitination related with DNA repair are involved in lung cancer pathogenesis, which may influence patient survival. We also conjecture the relationship among RNF20, RNF40 and TRAIP during the DDR pathway. Therefore, future studies to investigate the role of TRAIP in DDR and its expression patterns related with other DDR molecules such as RNF20 and RNF40 in cancer patient samples are warranted to elucidate the detailed mechanisms of DDR and its dysregulation in cancer and then to pave the way for developing novel therapeutic interventions.

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## Availability of data and materials

All data generated or analyzed during this study are included in this published article.

## Authors' contributions

YGH, MY and MC performed the experiments, analyzed the data and drafted the manuscript. SGL and HK designed and interpreted the study, and wrote and edited the manuscript. All authors read and approved the manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

## References

1. Durocher D and Jackson SP: DNA-PK, ATM and ATR as sensors of DNA damage: Variations on a theme? Curr Opin Cell Biol 13: 225-231, 2001.
2. Kerzendorfer C and O'Driscoll M: Human DNA damage response and repair deficiency syndromes: Linking genomic instability and cell cycle checkpoint proficiency. DNA Rep 8: 1139-1152, 2009.
3. Uckelmann M and Sixma TK: Histone ubiquitination in the DNA damage response. DNA Repair 56: 92-101, 2017.
4. Dantuma NP and van Attikum H: Spatiotemporal regulation of posttranslational modifications in the DNA damage response. EMBO J 35: 6-23, 2016.
5. Fuchs G, Shema E, Vesterman R, Kotler E, Wolchinsky Z, Wilder S, Golomb L, Pribluda A, Zhang F, Haj-Yahya M, *et al*: RNF20 and USP44 regulate stem cell differentiation by modulating H2B monoubiquitylation. Mol Cell 46: 662-673, 2012.
6. Minsky N, Shema E, Field Y, Schuster M, Segal E and Oren M: Monoubiquitinated H2B is associated with the transcribed region of highly expressed genes in human cells. Nat Cell Biol 10: 483-488, 2008.
7. Zhu B, Zheng Y, Pham AD, Mandal SS, Erdjument-Bromage H, Tempst P and Reinberg D: Monoubiquitination of human Histone H2B: The factors involved and their roles in *HOX* gene regulation. Mol Cell 20: 601-611, 2005.
8. Thorne AW, Sautiere P, Briand G and Crane-Robinson C: The structure of ubiquitinated Histone H2B. EMBO J 6: 1005-1010, 1987.
9. Weake VM and Workman JL: Histone ubiquitination: Triggering gene activity. Mol Cell 29: 653-663, 2008.
10. Shanbhag NM, Rafalska-Metcalf IU, Balane-Bolivar C, Janicki SM and Greenberg RA: ATM-dependent chromatin changes silence transcription in cis to DNA double-strand breaks. Cell 141: 970-981, 2010.
11. Xu Z, Song Z, Li G, Tu H, Liu W, Liu Y, Wang P, Wang Y, Cui X, Liu C, *et al*: H2B ubiquitination regulates meiotic recombination by promoting chromatin relaxation. Nucleic Acids Res 44: 9681-9697, 2016.
12. Pavri R, Zhu B, Li G, Trojer P, Mandal S, Shilatifard A and Reinberg D: Histone H2B monoubiquitination functions cooperatively with FACT to regulate elongation by RNA polymerase II. Cell 125: 703-717, 2006.
13. Zhang XY, Varthi M, Sykes SM, Phillips C, Warzecha C, Zhu W, Wyce A, Thorne AW, Berger SL and McMahon SB: The putative cancer stem cell marker USP22 is a subunit of the human SAGA complex required for activated transcription and cell-cycle progression. Mol Cell 29: 102-111, 2008.

14. Shema E, Tirosh I, Aylon Y, Huang J, Ye C, Moskovits N, Raver-Shapira N, Minsky N, Pirngruber J, Tarcic G, *et al*: The Histone H2B-specific ubiquitin ligase RNF20/hBRE1 acts as a putative tumor suppressor through selective regulation of gene expression. Genes Dev 22: 2664-2676, 2008.
15. Moyal L, Lerenthal Y, Gana-Weisz M, Mass G, So S, Wang SY, Eppink B, Chung YM, Shalev G, Shema E, *et al*: Requirement of ATM-dependent monoubiquitylation of Histone H2B for timely repair of DNA double-strand breaks. Mol Cell 41: 529-542, 2011.
16. Lee SY and Choi Y: TRAF-interacting protein (TRIP): A novel component of the tumor necrosis factor receptor (TNFR)-and CD30-TRAF signaling complexes that inhibits TRAF2-mediated NF-kappaB activation. J Exp Med 185: 1275-1286, 1997.
17. Rothe M, Pan MG, Henzel WJ, Ayres TM and Goeddel DV: The TNFR2-TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. Cell 83: 1243-1252, 1995.
18. Besse A, Campos AD, Webster WK and Darnay BG: TRAF-interacting protein (TRIP) is a RING-dependent ubiquitin ligase. Biochem Biophys Res Commun 359: 660-664, 2007.
19. Regamey A, Hohl D, Liu JW, Roger T, Kogerman P, Toftgard R and Huber M: The tumor suppressor CYLD interacts with TRIP and regulates negatively nuclear factor kappaB activation by tumor necrosis factor. J Exp Med 198: 1959-1964, 2003.
20. Zhou Q and Geahlen R: The protein-tyrosine kinase Syk interacts with TRAF-interacting protein TRIP in breast epithelial cells. Oncogene 28: 1348-1356, 2009.
21. Deshaies RJ and Joazeiro CA: RING domain E3 ubiquitin ligases. Annu Rev Biochem 78: 399-434, 2009.
22. Chapard C, Meraldi P, Gleich T, Bachmann D, Hohl D and Huber M: TRAIP is a regulator of the spindle assembly checkpoint. J Cell Sci 127: 5149-5156, 2014.
23. Harley ME, Murina O, Leitch A, Higgs MR, Bicknell LS, Yigit G, Blackford AN, Zlatanou A, Mackenzie KJ, Reddy K, *et al*: TRAIP promotes DNA damage response during genome replication and is mutated in primordial dwarfism. Nat Genet 48: 36-43, 2016.
24. Feng W, Guo Y, Huang J, Deng Y, Zang J and Huen MS: TRAIP regulates replication fork recovery and progression via PCNA. Cell Discov 2: 16016, 2016.
25. Soo Lee N, Jin Chung H, Kim HJ, Yun Lee S, Ji JH, Seo Y, Hun Han S, Choi M, Yun M, Lee SG, *et al*: TRAIP/RNF206 is required for recruitment of RAP80 to sites of DNA damage. Nat Commun 7: 10463, 2016.
26. Hoffmann S, Smedegaard S, Nakamura K, Mortuza GB, Räschle M, Ibañez de Opakua A, Oka Y, Feng Y, Blanco FJ, Mann M, *et al*: TRAIP is a PCNA-binding ubiquitin ligase that protects genome stability after replication stress. J Cell Biol 212: 63-75, 2016.
27. Kim H, Huang J and Chen J: CCDC98 is a BRCA1-BRCT domain-binding protein involved in the DNA damage response. Nat Struct Mol Biol 14: 710-715, 2007.
28. Kim H, Chen J and Yu X: Ubiquitin-binding protein RAP80 mediates BRCA1-dependent DNA damage response. Science 316: 1202-1205, 2007.
29. Lorick KL, Jensen JP, Fang S, Ong AM, Hatakeyama S and Weissman AM: RING fingers mediate ubiquitin-conjugating enzyme (E2)-dependent ubiquitination. Proc Natl Acad Sci USA 96: 11364-11369, 1999.
30. Jemal A, Bray F, Center MM, Ferlay J, Ward E and Forman D: Global cancer statistics. CA Cancer J Clin 61: 69-90, 2011.
31. Dela Cruz CS, Tanoue LT and Matthay RA: Lung cancer: Epidemiology, etiology, and prevention. Clin Chest Med 32: 605-644, 2011.
32. Molina JR, Yang P, Cassivi SD, Schild SE and Adjei AA: Non-small cell lung cancer: Epidemiology, risk factors, treatment, and survivorship. Mayo Clin Proc 83: 584-594, 2008.
33. Jung Y and Lippard SJ: Direct cellular responses to platinum-induced DNA damage. Chem Rev 107: 1387-1407, 2007.
34. Pearl LH, Schierz AC, Ward SE, Al-Lazikani B and Pearl FM: Therapeutic opportunities within the DNA damage response. Nat Rev Cancer 15: 166-180, 2015.
35. Jackson SP and Bartek J: The DNA-damage response in human biology and disease. Nature 461: 1071-1078, 2009.
36. European Medicines Agency: Lynparza recommended for approval in ovarian cancer. https://www.ema.europa.eu/en/news/lynparza-recommended-approval-ovarian-cancer.
37. Chen S, Jing Y, Kang X, Yang L, Wang DL, Zhang W, Zhang L, Chen P, Chang JF, Yang XM, *et al*: Histone H2B monoubiquitination is a critical epigenetic switch for the regulation of autophagy. Nucleic Acids Res 45: 1144-1158, 2017.
38. Jackson SP and Durocher D: Regulation of DNA damage responses by ubiquitin and SUMO. Mol Cell 49: 795-807, 2013.
39. Schwertman P, Bekker-Jensen S and Mailand N: Regulation of DNA double-strand break repair by ubiquitin and ubiquitin-like modifiers. Nat Rev Mol Cell Biol 17: 379-394, 2016.
40. Mattiroli F, Vissers JH, van Dijk WJ, Ikpa P, Citterio E, Vermeulen W, Marteijn JA and Sixma TK: RNF168 ubiquitinates K13-15 on H2A/H2AX to drive DNA damage signaling. Cell 150: 1182-1195, 2012.
41. Kalb R, Mallery DL, Larkin C, Huang JT and Hiom K: BRCA1 is a histone-H2A-specific ubiquitin ligase. Cell Rep 8: 999-1005, 2014.
42. Wood A, Krogan NJ, Dover J, Schneider J, Heidt J, Boateng MA, Dean K, Golshani A, Zhang Y, Greenblatt JF, *et al*: Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. Mol Cell 11: 267-274, 2003.
43. Wang H, Wang L, Erdjument-Bromage H, Vidal M, Tempst P, Jones RS and Zhang Y: Role of histone H2A ubiquitination in polycomb silencing. Nature 431: 873-878, 2004.
44. Wallace HA, Merkle JA, Yu MC, Berg TG, Lee E, Bosco G and Lee LA: TRIP/NOPO E3 ubiquitin ligase promotes ubiquitylation of DNA polymerase η. Development 141: 1332-1341, 2014.