Critical Roles of Ring Finger Protein RNF8 in Replication Stress Responses

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Histone ubiquitylation is emerging as an important protective component in cellular responses to DNA damage. The ubiquitin ligases RNF8 and RNF168 assemble ubiquitin chains onto histone molecules surrounding DNA breaks and facilitate retention of DNA repair proteins. Although RNF8 and RNF168 play important roles in repair of DNA double strand breaks, their requirement for cell protection from replication stress is largely unknown. In this study, we uncovered RNF168-independent roles of RNF8 in repair of replication inhibition-induced DNA damage. We showed that RNF8 depletion, but not RNF8 depletion, hyper-sensitized cells to hydroxyurea and aphidicolin treatment. Consistently, hydroxyurea-induced persistent DNA lesions and sustained CHK1 activation in RNF8-depleted cells. In line with strict requirement for RAD51-dependent repair of hydroxyurea-stalled replication forks, RNF8 depletion compromised RAD51 accumulation onto single strand DNA lesions and sustained CHK1 activation in RNF8-depleted cells. In total, our study highlights the differential requirement for the ubiquitin ligase RNF8 in facilitating repair of replication stress-associated DNA damage.

The ubiquitin-dependent DNA-damage signaling cascade involving the E3 ubiquitin ligases RNF8 and RNF168 plays key roles in coordinating cell cycle progression and DNA repair (1–8). Dysregulation of this cascade contributes to genome instability and tumorigenesis (9, 10). Current evidence suggests that, in concert with the E2-conjugating enzyme UBC13, RNF168 amplifies the RNF8-initiated DNA-damage signal, in part, by extending non-degradative Lys63-linked ubiquitin chains on H2A-type histone molecules. Ubiquitylated histones then serve as recruiting factors to promote productive assembly of checkpoint and repair proteins, including 53BP1, RAD51, and BRCA1, to the damaged-modified chromatin (11). However, despite the mechanistic details that ascribed crucial roles of the RNF8-RNF168 module for cell survival from ionizing radiation-induced DNA double strand breaks and for class-switch recombination (9, 12–14), it remains largely unknown whether the ubiquitin ligases are similarly required for repair of other types of DNA lesions.

Homologous recombination (HR) DNA repair factors play crucial roles following replication arrest in mammalian cells. Previous work indicated that HR-deficient cells, but not non-homologous end-joining (NHEJ)-defective cells, are especially sensitive to agents that induce replication fork stalling (15). Moreover, cells that overexpress the HR recombinase RAD51 rendered cells resistant to replication inhibitors, including hydroxyurea (HU) and thymidine (16), suggesting that HR-repair proteins play a predominant role in repairing restart of damaged replication forks. Interestingly, the E3 ubiquitin ligase RNF8 has been implicated in efficient HR DNA repair, in part, via the RAD18-RAD51C axis (17). Accordingly, RNF8 deficiency compromised RAD51 focal accumulation at IR-induced DNA breaks, and these cells exhibited impaired HR as determined using the mutant GFP gene conversion assay. By contrast, cells deficient in RNF168 displayed enhanced RAD51 foci formation in response to IR (10). More strikingly, whereas RNF8 is essential in IR-induced foci formation of RAD18, BRCA1, and 53BP1, RNF168-deficient cells supported double strand break association of RAD18 and BRCA1 at later time points after IR treatment (8, 10) (supplemental Fig. S1). Together, these data suggest that there may be functional differences between RNF8 and RNF168 in cellular responses to DNA damage.

**EXPERIMENTAL PROCEDURES**

**Antibodies**—The RNF168 polyclonal antibody was raised against MBP-RNF168 fusion protein and affinity-purified using a column coated with GST-RNF168 fusion protein. Antibodies specifically recognizing RNF8, H2AX, γH2AX, BRCA1, 53BP1, RAD18, RAP80, CtIP, RAD51, ubiquitin, phospho-H3 (H3S10P),...
and phospho-CHK1 were previously described (2, 17–20). Anti-CHK1 and anti-PCNA antibodies were obtained from Santa Cruz Biotechnology. Anti-BrDU antibodies were from Roche Applied Science. Anti-RPA1 and anti-RPA2 antibodies were from Upstate and Calbiochem, respectively. Anti-PARP1 antibodies were purchased from Dharmacon (2, 17, 20–23). siRNA transfection using Oligofectamine (Invitrogen) was performed as indicated. siRNAs targeting RNF8 (siRNA#1 + siRNA#2), 53BP1 (SMARTpool), RNF168 (SMARTpool), RAP80, RAD18, CtIP, RAD51, CHK1, and a non-targeting control siRNA were purchased from Dharmacon. Plasmid transfection was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol. Pooled siRNAs were used for all experiments unless otherwise specified. Plasmid transfection was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol.

Cell Culture, siRNA, and Transfection—Cell lines were cultured in DMEM supplemented with 10% FBS and maintained in 5% CO₂ at 37 °C. siRNAs targeting RNF8 (siRNA#1 + siRNA#2), 53BP1 (SMARTpool), RNF168 (SMARTpool), RAP80, RAD18, CtIP, RAD51, CHK1, and a non-targeting control siRNA were purchased from Dharmacon. siRNA transfection using Oligofectamine (Invitrogen) was performed according to the manufacturer’s protocol. Pooled siRNAs were used for all experiments unless otherwise specified. Plasmid transfection was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol.

Immunostaining Procedure—To visualize damage-induced foci, cells were cultured on coverslips and treated with indicated DNA-damaging agent. At indicated time, cells were washed in PBS, permeabilized in 0.5% Triton X-100 solution for 3 min at room temperature, and incubated in 3% paraformaldehyde for 20 min. Samples were blocked with 5% goat serum and then incubated with primary antibodies for 30 min. Samples were washed three times and incubated with secondary antibodies for 30 min. Cells were then stained with DAPI to visualize nuclear DNA. The coverslips were mounted onto glass slides with Antifade solution and visualized using a Nikon ECLIPSE E800 fluorescence microscope.

Clonogenic Survival Assay, G₂/M Checkpoint Assay, and Cell Cycle Analysis—Clonogenic survival assays were performed essentially as described previously (2). To quantify cells in mitosis during HU recovery, cells released from HU were incubated with 20 ng/ml nocodazole to prevent cells from progressing through mitosis. 12 h after HU release, cells were harvested, fixed with 70% ethanol, and immunostained with anti-phospho-H1 (H3S10P) antibodies. Processed cells were analyzed by flow cytometry, and the percentage of H3S10P-positive cells was quantified using WinMDI. The percentage of cells in each cell cycle phase was determined using ModFit software.

Chromatin Fractionation and Immunoprecipitation Experiments—Preparation of chromatin fractions were described previously with modifications (22). Briefly, cells were harvested at the indicated times after treatment and washed once with PBS. Cell pellets were subsequently resuspended in NETN (0.5% NP-40, 2 mM EDTA, 50 mM Tris-Cl, pH 8.0, and 100 mM NaCl) and protease inhibitors, soluble fraction) and incubated on ice for 10 min. The resulting pellet, enriched in chromatin-bound proteins, was resuspended in denaturing buffer and boiled. For immunoprecipitation using antibodies against H2A, H2AX, or γH2AX, cell pellets were resuspended in denaturing buffer (20 mM Tris, 50 mM NaCl, 0.5% Nonidet P-40, 0.5% deoxycholate, 0.5% SDS, and 1 mM EDTA) and sonicated briefly. Lysates were then incubated with protein A-agarose beads conjugated with antibodies at 4 °C overnight with gentle agitation. Beads were washed three times with denaturing buffer and proteins were eluted by boiling in sampling buffer.

RESULTS

RNF8 Promotes Replication Stress-induced Histone Ubiquitylation—Previous work has established that RNF8-UBC13 promotes local histone ubiquitylation at the vicinity of double strand breaks, which in turn recruits RNF168 to further amplify the DNA-damage signal. To test whether replication stress may also mount a similar cellular response, we first tested whether HU treatment induces such RNF8-RNF168-dependent histone ubiquitylation events. Indeed, similar to ionizing radiation treatment (Fig. 1a), cells challenged with the replication inhibitor HU accumulated significant amounts of ubiquitin-modified species of histone H2A and its variant H2AX (Fig. 1, b–d), suggesting that histone ubiquitylation is a conserved cellular event associated with DNA-damage responses. Moreover, consistent with ascribed roles of the E3 ubiquitin ligases RNF8-RNF168 in IR-induced histone ubiquitylation, in cells treated with HU, inactivation of RNF8 impaired γH2AX ubiquitylation, whereas mono-ubiquitin-modified γH2AX could be reproducibly detected in RNF168-depleted...
RNF8 Promotes Repair of Hydroxyurea-damaged Replication Forks

**FIGURE 2. RNF8 promotes cell survival upon replication stress.** a. Western blot showing expression of RNF8 and RNF168 in whole cell extracts derived from HeLa cells treated with indicated siRNAs. Asterisks denote nonspecific bands; b–d, clonogenic cell survival assays in RNF8-, RNF168-, or control (CTR)-siRNA treated cells. HeLa cells were transfected with indicated siRNAs twice at 24-h intervals. Cells were seeded onto 60-mm dishes and were irradiated (b) or incubated with hydroxyurea (HU; panel c) or aphidicolin (APH; panel d). Cells were cultured for 10 days before colonies were counted. Results represent mean ± S.E. of three experiments.

RNF8 Promotes Cell Survival in Response to Replication Stress—To explore whether RNF8 and RNF168 are similarly required for repair of replication stress-induced DNA damage, we first used clonogenic cell survival as readout. Consistent with previous work, we found that cells depleted of either RNF8 or RNF168 using pooled siRNAs sensitized cells to IR treatment (Fig. 2, a and b). Surprisingly, when we examined possible requirements for the E3 ubiquitin ligases in survival following replication stress, we observed notable differences in cells with RNF8 or RNF168 inactivation. In sharp contrast to RNF168 depletion, which did not noticeably affect cell survival compared with control cells, RNF8 deficiency resulted in significantly reduced survival rates in cells challenged with replication inhibitors HU and aphidicolin (Fig. 2, c and d). Consistently, direct requirement for the E3 ubiquitin ligases in cell protection against HU was also observed when pooled siRNAs deconvoluted into individual siRNAs were used to down-regulate RNF8 or RNF168 in HeLa cells (supplemental Fig. S2, a–d), as well as in cell lines deficient in RNF8 or RNF168 (supplemental Fig. S2, e and f). These data led us to speculate about a specific requirement for RNF8 in promoting cell survival in response to replication stress. We furthered our studies employing RNA interference in HeLa cells, which allowed direct comparison and analysis, in isogenic strains, of roles of RNF8 and RNF168 in promoting cell survival in response to replication stress.

Hydroxyurea Induces a Sustained CHK1-dependent G2 Checkpoint in RNF8-deficient Cells—Because there were no substantial difference in S-phase progression (supplemental Fig. S3a) or with parameters associated with DNA replication (i.e. BrdU uptake; supplemental Fig. S3b) among RNF8-, RNF168-, or control siRNA-treated cells, we monitored BrdU uptake as well as cell cycle progression of these cells after releasing them from a HU-induced early S-phase block. Accordingly, apart from a mild delay in S-phase completion in RNF8-depleted cells (Fig. 3a and supplemental Fig. S3, c and d), we found that RNF8 inactivation, in stark contrast to control and RNF168-depleted cells, resulted in prominent accumulation of cells at G2/M phase 12 h after release from HU (Fig. 3a). To test whether these cells accumulate at the G2-phase, we released cells from HU in the presence of nocodazole, which arrests cells at mitosis. Using the mitotic marker phospho-histone H3 (H3S10P), we quantified the percentage of cells in mitosis 12 h after HU release. Indeed, compared with RNF168- and control siRNA-treated cells, RNF8 depletion resulted in delay in mitotic entry (Fig. 3b).

Given the established role of the CHK1 kinase in activating the damage-induced G2/M checkpoint, we tested whether RNF8-depleted cells may be arrested at the CHK1-dependent G2 checkpoint. Indeed, not only did RNF8-depleted cells exhibit prolonged cells may be arrested at the CHK1-dependent G2 checkpoint. Indeed, not only did RNF8-depleted cells exhibit prolonged cells, which is consistent with the ascribed role of RNF8 in priming histone ubiquitylation at DNA breaks. Together, these data support the idea that the E3 ubiquitin ligases RNF8 and RNF168 may participate in cellular responses upon replication stress.

Persistence of HU-induced ssDNA Lesions in RNF8-deficient Cells—Our observation, that RNF8 is required for timely progression through the CHK1-dependent G2 checkpoint following replication arrest, suggests that RNF8 promotes repair of HU-damaged replication forks. HU treatment leads to the depletion of the cellular pool of dNTPs, uncouples replicative unwinding and DNA synthesis, and results in accumulation of ssDNA lesions. Using RPA2 foci formation as a surrogate marker for ssDNAs, we confirmed that HU treatment induced markedly elevated numbers of ssDNAs lesions, which did not require C-terminal binding protein interacting protein (CtIP)-dependent DNA resection (Fig. 4, a–c). To confirm that HU induces ssDNA gaps associated with DNA replication, we labeled cells with BrdU and performed co-immunostaining experiments using anti-BrdU and anti-proliferating cell nuclear antigen (PCNA) antibodies under non-denaturing conditions. We found that HU incubation greatly elevated the number of ssDNAs in RNF8-, RNF168-, and control siRNA-treated cells (Fig. 4d), indicating that these cells are equally sensitive to HU treatment. Strikingly, when we assayed BrdU positivity after HU release, we observed persistence of ssDNA lesions in RNF8-deficient cells as opposed to RNF168- and control siRNA-treated cells (Fig. 4e and supplemental Fig. S5a). A similar requirement for RNF8 was observed in the clearance of HU-induced RPA foci formation (Fig. 4, f and g, and supplemental Fig. S4b) as well as RPA enrichment on chromatins (Fig. 4h). Given that
RPA-coated ssDNAs play pivotal roles in ATR-CHK1 activation. We concluded that the sustained CHK1-dependent G2-checkpoint arrest observed in RNF8-depleted cells following HU recovery is attributed to RPA2-ssDNA accumulation in these cells.

**Impaired RAD51 Loading and Sustained DNA Damage Signaling with RNF8 Deficiency**—Phosphorylation of DNA-PK (DNA-PK pSer2056) closely correlates with engagement of NHEJ (24). Our observations that the NHEJ pathway was not substantially activated in response to HU treatment (Fig. 5a) and that HU elicited persistent ssDNA lesions in RNF8-depleted cells prompted us to speculate whether repair of damaged replication forks may be compromised in these cells. HR DNA-repair proteins, including the recombinase RAD51, have been implicated in proper repair/restart of stalled replication forks. Assimilation of RAD51 onto RPA-coated ssDNAs represents the initial event leading to HR repair. In line with previous work (14, 17), RNF8 depletion led to impaired RAD51 focal accumulation upon HU treatment (Fig. 5, b and c). Surprisingly, we observed little difference in RAD51 foci-forming ability in either RNF168-depleted cells or control cells, illustrating a strict requirement of RNF8 for proper loading of HR-repair machinery and repair of HU-induced DNA lesions. Consistent with the requirement of HR machinery for repair of HU-induced DNA lesions, inactivation of HR-promoting factors RAD18 and the RAP80-BRCA1 axis, but not 53BP1, similarly impaired RAD51 focal accumulation (Fig. 5, d and e). Given the prominent role of RAD18 in promoting RAD51 loading to HU-induced DNA lesions, we tested whether RAD18, and its ability to localize to RNF8-dependent ubiquitin structures at DNA-damage sites, are required for cell protection following HU treatment (17). Indeed, using clonogenic survival assay as readout, we found that both RAD18 and its zinc finger are important in optimal cell survival in response to HU (supplemental Fig. S6a), suggesting that the RAD18 plays a cru-

![FIGURE 3. Hydroxyurea induces prolonged G2 phase cell cycle arrest in RNF8-deficient cells.](image)
RNF8 Promotes Repair of Hydroxyurea-damaged Replication Forks

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RNF8 and RNF168 ubiquitin ligases orchestrate DNA-damage responses via a non-canonical ubiquitin-dependent signaling pathway (25). Specifically, RNF8-RNF168 catalyze histone ubiquitylation at chromatin domains flanking a DNA-damage site, facilitate the accumulation of checkpoint and repair factors, and promote DNA repair and cell survival. In contrast to their similar functional requirement for IR-induced or programmed double strand break repair (2–9, 12–14), our study uncovered a specific requirement for RNF8, but not RNF168, in the repair of replication-associated DNA damage. We found that, in response to HU treatment, RNF8 promoted RAD51-dependent repair of damaged replication forks, dysregulation of which resulted in sustained DNA damage, prolonged G2 arrest, and compromised cell survival.

EXPOSURE OF ssDNA LESIONS RESULTS IN ACCUMULATION AND SUBSEQUENT PHOSPHORYLATION OF RPA COMPLEXES, WHICH IN TURN SIGNALS FOR ASSIMILATION OF THE RECOMBINASE RAD51 ONTO ssDNAs. Our observation, that both RPA and CHK1 phosphorylation...
persisted in RNF8-depleted cells, is suggestive of defective DNA repair in these cells. Previous studies have implicated homologous recombination DNA repair factors, including RAD51, in repair and restart of damaged replication forks. Consistently, we found that RAD51 accumulation to HU-induced RPA-coated DNA lesions was impaired in RNF8- but not RNF168-depleted cells, suggesting that RNF8 promotes RAD51-dependent repair and/or restart of damaged replication forks. Although we propose that RNF168 is largely dispensable for cell survival following replication inhibition, it is noteworthy to mention that proper RAD18 accumulation at DNA-damage sites requires the concerted actions of RNF8 and RNF168 (supplemental Fig. S1). Interestingly, we found that RNF168-deficient RIDDLE cells, unlike those with RNF8 deficiency, supported RAD18 foci formation 24 h post IR treatment. Our observation that RAD18, in the absence of RNF168, is recruited to DNA-damage sites with reduced kinetics implies that RNF8 catalyzes “limiting” amounts of ubiquitin conjugates, which over time accumulates to levels such that microscopically visible RAD18 foci become detectable. Although it remains to be seen whether mono-ubiquitylated H2A-type histones are responsible for tethering RAD18 to the vicinity of DNA lesions (Fig. 1e), given the fact that both RAD18 and its ability to associate at RNF8-dependent ubiquitin structures were pivotal in promoting cell survival in response to HU treatment, and that RNF8 and RAD18 are epistatic in cellular response to genotoxic stress, we speculate that RNF8 promotes replication fork repair by concentrating repair factors, including RAD18 and RAD51, at HU-induced ssDNA lesions.

Interestingly, our observation indicated that levels of damage-induced, RNF8-dependent H2AX mono-ubiquitylation were significantly reduced in cells depleted of RNF168. This observation can be explained by roles of RNF168 in amplifying the ubiquitin-dependent DNA-damage signals at the vicinity of
DNA breaks. Apart from “extending” the RNF8-primed histone ubiquitylation via K63-linked poly-ubiquitylation, docking of RNF168 to the RNF8-primed ubiquitylated H2A-type histones may allow it to “spread” DNA-damage signals by mono-ubiquitylating adjacent histone molecules. In support of the possibility that both RNF8 and RNF168 may promote H2AX mono-ubiquitylation, a previous study indicated that co-depletion of both E3 ubiquitin ligases is essential to inhibit the ATM-mediated gene silencing effect near a DNA double strand break (26). Further experiments will be needed to understand the RNF8-and RNF168-catalyzed ubiquitin conjugates and their topologies at sites of DNA damage.

In summary, our study uncovered distinct and strict requirement for RNF8 in cell recovery following replication arrest. Notably, we found that RNF8 alone was sufficient in promoting timely repair of HU-induced DNA damage, in part, via RAD51-dependent replication fork repair. Given the dynamic nature and the complexity of histone ubiquitylation at sites of DNA breaks, it remains to be seen whether and how ubiquitin signals may have evolved with more sophisticated and specific tasks in the maintenance of genome stability.

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REFERENCES

1. Marteijn, J. A., Bekker-Jensen, S., Mailand, N., Lans, H., Schwertman, P., Gourdin, A. M., Dantuma, N. P., Lukas, J., and Vermeulen, W. (2009) J. Cell Biol. 186, 835–847
2. Huen, M. S., Grant, R., Manke, I., Minn, K., Yu, X., Yaffe, M. B., and Chen, J. (2007) Cell 131, 901–914
3. Kolas, N. K., Chapman, J. R., Nakada, S., Yanko, J., Chahwan, R., Sweeney, F. D., Panier, S., Mendez, M., Wildenhain, J., Thomson, T. M., Pelletier, L., Jackson, S. P., and Durocher, D. (2007) Science 318, 1637–1640
4. Mailand, N., Bekker-Jensen, S., Fastrup, H., Melander, F., Bartek, J., Lukas, C., and Lukas, J. (2007) Cell 131, 887–900
5. Wang, B., and Elledge, S. J. (2007) Proc. Natl. Acad. Sci. U.S.A. 104, 20759–20763
6. Doil, C., Mailand, N., Bekker-Jensen, S., Menard, P., Larsen, D. H., Pepperkok, R., Ellenberg, J., Panier, S., Durocher, D., Bartek, J., Lukas, C., and Lukas, J. (2009) Cell 136, 435–446
7. Pinato, S., Scandiuzzi, C., Arnaudo, N., Citterio, E., Gaudino, G., and Penengo, L. (2009) BMC Mol. Biol. 10, 55
8. Stewart, G. S., Panier, S., Townsend, K., Al-Hakim, A. K., Kolas, N. K., Miller, E. S., Nakada, S., Yanko, J., Olivarius, S., Mendez, M., Oldreive, C., Wildenhain, J., Tagliaferro, A., Pelletier, L., Taubenheim, N., Durandy, A., Byrd, P. J., Stankovic, T., Taylor, A. M., and Durocher, D. (2009) Cell 136, 420–434
9. Li, L., Halaby, M. J., Hakem, A., Cardoso, R., El Ghamrasni, S., Harding, S., Chan, N., Bristow, R., Sanchez, O., Durocher, D., and Hakem, R. (2010) J. Exp. Med. 207, 983–997
10. Stewart, G. S., Stankovic, T., Byrd, P. J., Wechsler, T., Miller, E. S., Huissoon, A., Drayson, M. T., West, S. C., Elledge, S. J., and Taylor, A. M. (2007) Proc. Natl. Acad. Sci. U.S.A. 104, 16910–16915
11. Huen, M. S., and Chen, J. (2010) Trends Biochem. Sci. 35, 101–108
12. Noon, A. T., Shibata, A., Rief, N., Lőbrich, M., Stewart, G. S., Jeggo, P. A., and Goodarzi, A. A. (2010) Nat. Cell Biol. 12, 177–184
13. Ramachandran, S., Chahwan, R., Nepal, R. M., Frieder, D., Panier, S., Roa, S., Zaheen, A., Durocher, D., Scharff, M. D., and Martin, A. (2010) Proc. Natl. Acad. Sci. U.S.A. 107, 809–814
14. Santos, M. A., Huen, M. S., Jankovic, M., Chen, H. T., López-Contreras, A. J., Klein, I. A., Wong, N., Barbancho, J. L., Fernandez-Capetillo, O., Nussenzweig, M. C., Chen, J., and Nussenzweig, A. (2010) J. Exp. Med. 207, 973–981
15. Lundin, C., Erixon, K., Arnaudeau, C., Schultz, N., Jenssen, D., Meuth, M., and Helleday, T. (2002) Mol. Cell. Biol. 22, 5869–5878
16. Lundin, C., Schultz, N., Arnaudeau, C., Mohnhinda, A., Hansen, L. T., and Helleday, T. (2003) J. Mol. Biol. 328, 521–535
17. Huang, J., Huen, M. S., Kim, H., Leung, C. C., Glover, J. N., Yu, X., and Chen, J. (2009) Nat. Cell Biol. 11, 592–603
18. Sy, S. M., Huen, M. S., Zhu, Y., and Chen, J. (2009) J. Biol. Chem. 284, 18302–18310
19. Yu, X., Chini, C. C., He, M., Mer, G., and Chen, J. (2003) Science 302, 639–642
20. Kim, H., Chen, J., and Yu, X. (2007) Science 316, 1202–1205
21. Huen, M. S., Huang, J., Yuan, J., Yamamoto, M., Akira, S., Ashley, C., Xiao, W., and Chen, J. (2008) Mol. Cell. Biol. 28, 6104–6112
22. Huen, M. S., Huang, J., Leung, J. W., Sy, S. M., Leung, K. M., Ching, Y. P., Tsao, S. W., and Chen, J. (2010) Mol. Cell 37, 854–864
23. Yuan, J., and Chen, J. (2009) J. Biol. Chem. 284, 31746–31752
24. Chen, B. P., Chan, D. W., Kobayashi, J., Burma, S., Asaithamby, A., Morotomi-Yano, K., Botvinick, E., Qin, J., and Chen, D. J. (2005) J. Biol. Chem. 280, 14709–14715
25. Panier, S., and Durocher, D. (2009) DNA Repair (Amst.) 8, 436–443
26. Shanbhag, N. M., Rafalska-Metcalf, I. U., Balane-Bolivar, C., Janicki, S. M., and Greenberg, R. A. (2010) Cell 141, 970–981