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Ren, Cong
Cho, Seong-Jun
Jung, Yong-Sam
et al.

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DNA polymerase eta is regulated by poly(rC)-binding protein 1 via mRNA stability

Cong Ren*, Seong-Jun Cho*, Yong-Sam Jung*, and Xinbin Chen*¹

*Comparative Oncology Laboratory, Schools of Medicine and Veterinary Medicine, University of California at Davis, Davis, CA 95616

Abstract

DNA polymerase eta (POLH), a target of p53 tumor suppressor, plays a key role in translesion DNA synthesis (TLS). Loss of POLH is responsible for human cancer prone syndrome, Xeroderma Pigmentosum Variant (XPV). Due to its critical role in DNA repair and genome stability, POLH expression and activity are regulated by multiple pathways. In this study, we found that the levels of both *POLH* transcript and protein were decreased upon knockdown of the transcript encoding poly(rC)-binding protein 1 (PCBP1). We also found that the half-life of *POLH* mRNA was markedly decreased upon knockdown of PCBP1. Moreover, we found that PCBP1 directly bound to *POLH* 3'UTR and the PCBP1-binding site in *POLH* mRNA is an atypical AU-rich element. Finally, we showed that the AU-rich element in *POLH* 3'UTR was responsive to PCBP1 and sufficient for PCBP1 to regulate *POLH* expression. Altogether, we uncovered a novel mechanism by which *POLH* expression is controlled by PCBP1 via mRNA stability.

Keywords

POLH; p53; RNA binding protein; PCBP1; mRNA decay; AU-rich element

INTRODUCTION

DNA polymerase eta (POLH), a member of the Y-family DNA polymerases, is necessary for repair of DNA lesions induced by ultraviolet irradiation and carcinogens via translesion DNA synthesis (TLS) [1–6]. POLH can accurately repair cyclobutane pyrimidine dimers (CPDs), pyrimidine (6-4) pyrimidone photoadducts, 8,5'-cyclopurine-2'-deoxynucleosides (cPus) and 7,8-dihydro-8-oxoguanine (8-oxoG) caused by UV-irradiation or oxidative stress [7–11]. Upon DNA damage, POLH can be recruited to the sites of replication fork stalling by interacting with FANCD2 and PCNA [12–14]. Mutation of the *POLH* gene is associated with human syndrome, Xeroderma Pigmentosum Variant (XPV) [15–17]. XPV patients are prone to skin cancer [18–20]. Consistently, repression of *POLH* expression is observed in various types of skin cancer [18]. In addition to its role in TLS, POLH is necessary for

¹To whom correspondence should be addressed: Xinbin Chen, School of Veterinary Medicine, University of California at Davis, 1 Shields Ave, Davis, CA 95616; Tel.: +1 530 754 8404; Fax: +1 530-752-6042; xbchen@ucdavis.edu.

AUTHOR CONTRIBUTION

R.C. and X.C. designed the research, analyzed the data and wrote the manuscript; R.C. performed experiments; S.-J. C. and Y.-S. J. contributed reagents and analyzed the data.

hypermethylation of immunoglobulin genes [21, 22] and for maintenance of genome stability [23–26].

POLH expression is found to be regulated by multiple mechanisms, including transcriptional regulation by DNA damage in a p53-dependent manner [25] and protein stability by Pirh2 and Mdm2 E3 ligases [27, 28]. *C. elegans* POLH is targeted for proteasomal degradation upon SUMOylation by the Cul4-Ddb1-Cdt2 pathway [29]. Additionally, the enzymatic activity of POLH is regulated by posttranslational modifications, such as SUMOylation and monoubiquitination [30, 31]. In this study, we found that *POLH* expression is regulated by poly(rC)-binding protein 1 (PCBP1, also called heterogeneous nuclear ribonucleoprotein E1 (hnRNP E1) or α -CP1) via mRNA stability. We also found that PCBP1 directly binds to *POLH* 3'UTR. Interestingly, we found that an AU-rich element in *POLH* mRNA is recognized by and responsive to PCBP1 although several PCBP1-binding sites are CU-rich elements or oligo(rC) elements [32–35]. Together, we uncovered a novel mechanism by which *POLH* expression is regulated by PCBP1 via mRNA stability.

EXPERIMENTAL PROCEDURE

Cell culture

Human pancreatic cancer cell line MIA-PaCa2, human colon cancer cell line p53^{-/-} HCT116, human cervical carcinoma cell line ME180 and human breast cancer cell line MCF7 were cultured in DMEM (Invitrogen) with 10% fetal bovine serum (Hyclone) and maintained at 37°C in 5% CO₂ incubator.

Plasmid

Lentiviral vectors (pLKO.1-puro) expressing shRNA targeting luciferase and PCBP1 were purchased from Sigma Inc. The targeting sequences are 5'-CGTGAGTACTTCGAAATGTC-3' for control luciferase shRNA and 5'-CCCATGATCCAACACTGTGTAAT-3' (shPCBP1) or GCTCCTCTGGTAGGCAGGTTACT (shPCBP1*) for PCBP1 shRNA. pGEX-4T-3 plasmid was used to express GST and GST-fused PCBP1 proteins as previously described [36]. To generate mutant p53(R175H) reporter vector, the DNA fragments amplified from *POLH* 3'UTR were digested with *XhoI* and *NheI* and then ligated into pcDNA3-p53(R175H) vector [25] cut by *XhoI* and *XbaI*. The primers used for amplification of *POLH* 3' UTR are listed in Table 1. Plasmid RP11-22I24 (BACPAC Resources, Children's Hospital and Research Center at Oakland, CA), which carries the *POLH* locus, was used as a template to amplify *POLH* 3'UTR.

RNA interference

For lentivirus preparation, shRNA-expressing vector (10 μ g) and packaging plasmids (pMDL g/p RRE (5 μ g), pCMV-VSVG (5 μ g) and pRSV-REV (5 μ g)) were co-transfected into HEK 293T cells (6×10^6) using Expressfect™ transfection reagent (Denville Scientific). Lentiviral particles were collected from the medium every 24 h for 2 days and then filtered and concentrated by ultracentrifugation at 107,000 g in a Beckman SW41TI rotor for 2 h at 4°C. Cells were transduced with concentrated lentiviral particles and then treated with puromycin for 3 days to eliminate untransduced cells. For MCF7 and p53^{-/-} HCT116 cells,

1 μ g/ml of puromycin was used whereas 0.5 μ g/ml of puromycin was used for MIA-PaCa2 and ME180 cells.

Antibodies and western blot analysis

Mouse anti-PCBP1 (E-2), mouse anti-p63 (4A4) and rabbit anti-POLH (H-300) purchased from Santa Cruz were used for western blots. Rabbit anti-PCBP1 (catalog#8534) from cell signaling was used for immunoprecipitation. Mouse anti-HA antibody was purchased from Covance. Rabbit anti-actin was purchased from Sigma.

Whole cell lysates were prepared with 2 \times SDS sample buffer and separated in 8–10% SDS-PAGE, transferred to nitrocellulose membrane and then probed with primary and secondary antibodies, followed by chemiluminescent detection.

RNA isolation and RT-PCR

Total RNAs were extracted from cells using Trizol reagent (Invitrogen) according to the manufacturer's protocol. cDNA was synthesized using M-MLV reverse transcriptase (Promega) according to the manufacturer's manual. Semi-quantitative PCR was performed with GoTaq DNA polymerase (Promega). RT-qPCR was performed with Maxima SYBR Green qPCR Master Mix (Thermo) and the relative expression level was calculated upon normalization to the level of actin transcript. The sequences of primers used for PCR are listed in Table 1.

RNA immunoprecipitation assay (RNA-IP)

RNA-IP was carried out as previously described [37]. Briefly, $\sim 3 \times 10^6$ cells were lysed with 1 mL lysis buffer (10 mM HEPES, pH 7.0, 100 mM KCl, 100 mM NaCl, 10 mM MgCl₂, 0.5% NP-40, 1 mM DTT) supplemented with RiboLock™ ribonuclease inhibitor (Thermo Scientific) and protease inhibitor cocktails (Sigma). The cell lysates were centrifuged for 10 min at 13,000 rpm at 4°C, followed by immunoprecipitation with 2 μ g of rabbit anti-PCBP1 antibody or isotype control IgG at 4°C for 6 hours. The RNA-protein immunocomplexes were brought down by protein A-agarose beads (Sigma), followed by RT-PCR analysis.

RNA electrophoretic mobility shift assay (REMSA)

The probes used for REMSA were labeled during *in vitro* transcription of a DNA fragment containing the T7 promoter and a part or all of *POLH* 3'UTR. Briefly, 250 ng of purified DNA fragments was incubated with 20 μ Ci of [α -³²P]-UTP (800 Ci/mL, PerkinElmer), 0.5 mM each of rNTP (A, G and C), 10 U of T7 RNA polymerase (Ambion) and 20 U of RNase inhibitor (Thermo) in 10 μ L of reaction mixture at 37°C for 1 h. One unit of DNase I (Promega) was added to the reaction mixture to remove the DNA template. The labeled RNA probes were purified by Sephadex G-50 column to remove unlabeled free nucleotides. The radioactivity of probes was measured by a liquid scintillation counter. REMSA was performed as previously described [36]. Briefly, 50,000 CPM of [α -³²P]-labeled RNA probe, 250 nM GST or GST-PCBP1, and 100 ng/ μ L of yeast tRNA were mixed in 20 μ L of binding buffer (10 mM Tris-Cl, pH 8.0, 25 mM KCl, 10 mM MgCl₂ and 1 mM DTT) at room temperature for 20 min, followed by treatment with 100 U of RNase T1 (Ambion) for

15 min at 37°C to digest unprotected RNA fragments. The RNA-protein complexes were then separated in 7% native polyacrylamide gel and visualized by autoradiography.

RESULTS

***POLH* expression is decreased by knockdown of PCBP1**

A transcript with a long 3'UTR is often subject to posttranscriptional regulation, including mRNA stability. Since *POLH* has a long 3'UTR (~6,000 nt) along with several CU- and AU-rich elements, we examined whether PCBP1, a poly(rC)-binding protein, regulates *POLH* expression. To test this, PCBP1 was knocked down in MCF7 cells transduced with lentivirus expressing PCBP1 shRNAs for 3 days. A lentivirus expressing shRNA targeting luciferase mRNA was used as a negative control. We found that the level of POLH protein in MCF7 cells, which carries wild-type p53, was decreased by knockdown of PCBP1, but not control shRNA (Fig. 1A). To rule out potential effects of wild-type p53 on expression of *POLH*, a target of p53 [25], PCBP1 was knocked down in p53^{-/-} HCT116 cells, MIA-PaCa2 cells which carry a mutant p53 [38], and ME180 cells which express undetectable wild-type p53 [39]. We showed that knockdown of PCBP1 led to decreased expression of *POLH* regardless of the status of the p53 gene (Fig. 1B–D). As a control, Np63 in ME180 cells and TAp63 in MIA-PaCa2 cells were decreased by knockdown of PCBP1, consistent with a previous report [36]. Next, we examined whether the decreased levels of POLH protein are due to decreased levels of *POLH* transcript. Indeed, we found that the levels of *POLH* transcript were decreased in cells by knockdown of PCBP1 regardless of the status of the p53 gene (Fig. 2A–D). Together, these results suggest that PCBP1 is necessary for appropriate expression of *POLH*.

***POLH* mRNA stability is regulated by PCBP1**

As an RNA-binding protein, PCBP1 may bind to its target and then regulate the target's mRNA stability. To test this, the half-life of *POLH* mRNA was measured in p53^{-/-} HCT116 cells transduced with a lentivirus expressing luciferase shRNA or PCBP1 shRNA (shPCBP1) for 3 days. The cells were then treated with 100 μM 5, 6-Dichloro-1-β-D-ribofuranosylbenzimidazole (DRB), a transcription inhibitor, to block *de novo* RNA synthesis. We found that the half-life of *POLH* mRNA was decreased from ~2.57 h in control cells to ~1.44 h in PCBP1-knockdown cells (Fig. 3).

To examine whether PCBP1 physically associates with *POLH* mRNA *in vivo*, RNA immunoprecipitation assay followed by RT-PCR was performed with MIA-PaCa2, p53^{-/-} HCT116 and ME180 cells. We found that the level of *POLH* mRNA was highly enriched in anti-PCBP1 immunocomplexes (Fig. 4A–C). The levels of TAp63 α and Np63 α transcripts were also examined as positive controls and found to be enriched in anti-PCBP1 immunocomplexes (Fig. 4A–C), consistent with a previous report [36]. In contrast, no interaction was found between actin transcript and PCBP1 (Fig. 4).

***POLH* 3'UTR is recognized by and responsive to PCBP1**

To identify a region in *POLH* 3'UTR that is responsive to PCBP1, we generated seven reporter (mutant p53 R175H) plasmids which carry none or a segment from *POLH* 3'UTR

(Fig. 5A). Mutant p53 R175H was chosen as a reporter since mutant p53 is highly stable and can be easily detected. These reporters were expressed in MIA-PaCa2 cells transduced with a lentivirus expressing shRNA (shPCBP1) against luciferase or PCBP1. As expected, knockdown of PCBP1 led to decreased expression of endogenous *POLH* (Fig. 5B). We also found that knockdown of PCBP1 was capable of decreasing the level of mutant p53 protein from a reporter vector that contains the full-length or fragment A of *POLH* 3'UTR (Fig. 5B, 3'UTR and A panels). In contrast, knockdown of PCBP1 had no obvious effect on the expression of mutant p53 from the control vector that does not carry *POLH* 3'UTR and vectors that contain fragments B–E of *POLH* 3'UTR (Fig. 5B, Ctrl and B–E panels). Thus, fragment A (nt 2447–3607) of *POLH* 3'UTR carries a PCBP1-responsive element.

To define the PCBP1-responsive element in *POLH* 3'UTR, REMSA was performed with five RNA probes (A–E), which span the entire *POLH* 3'UTR, to map the binding site of PCBP1 in *POLH* transcript (Fig. 6A). p63 3'UTR was used as a positive control [36]. We showed that GST-fused PCBP1 bound strongly to fragment A and p63 probe, weakly to fragment C, but little if any to fragments B, D, and E (Fig. 6B). As expected, GST alone didn't bind to these probes (Fig. 6B). The specificity was confirmed by competition assay. As indicated in Fig. 6C, the binding of PCBP1 to fragment A was inhibited by addition of an excess amount of un-labeled fragment A or p63 RNA probe. Next, we prepared six sub-fragments from fragment A: A1 (nt 2447–2964); A2 (nt 2945–3607); A11 (nt 2447–2723); A12 (nt 2704–2964); A111 (nt 2447–2587); A112 (nt 2564–2723) (Fig. 6A). We showed that GST-fused PCBP1 bound to A1, A11, and A111, but not to A2, A12, and A112 (Fig. 6D–F). These results suggest that sub-fragment A111 (nt 2447–2587) contains the PCBP1-binding site.

PCBP1 directly binds to an AU-rich element in *POLH* 3'UTR

PCBP proteins are shown to preferentially recognize CU/C-rich elements [33]. Thus, we searched for such elements in A11 region (nt 2447–2723) of *POLH* 3'UTR. We found two well-conserved AU-rich elements, ARE-A (nt 2472–2492) and ARE-B (nt 2500–2522), but no CU/C-rich elements (Fig. 7A). Since PCBP1 is also found to recognize a poly(rU) element [40], we examined whether one or both ARE elements are recognized by PCBP1. To test this, we generated three RNA probes in which one or both ARE elements were deleted: ARE-A, ARE-B and ARE-AB (Fig. 7A). We found that ARE-A was still recognized by PCBP1 whereas the binding of PCBP1 to ARE-B and ARE-AB was markedly decreased (Fig. 7B), suggesting that ARE-B is the primary PCBP1-binding site. To further define the PCBP1 responsive element in *POLH* mRNA, we tested whether the poly(rU) sequence in ARE-B is required for PCBP1 binding. To address this, two RNA probes were generated: ARE-A-U2A and ARE-B-U2A in which U to A substitutions were made (Fig. 7C). REMSA was performed and showed that the binding of PCBP1 to probe ARE-B-U2A was nearly abolished (Fig. 7D). In contrast, the binding of PCBP1 to probe ARE-A-U2A was not decreased, but instead increased (Fig. 7D). These results suggest that the poly (rU) sequence in ARE-B is essential for PCBP1 binding.

To determine whether ARE-B is responsive to PCBP1 *in vivo*, we generated four additional reporter plasmids carrying mutant p53 R175H and a portion of *POLH* 3'UTR (nt 2447–nt

2723) with or without ARE-A, ARE-B, or both (Fig. 7E). These reporter plasmids were expressed in MIA-PaCa2 cells transduced with a lentivirus expressing shRNA against luciferase or PCBP1. As a positive control, the level of endogenous POLH was measured and found to be decreased upon knockdown of PCBP1 (Fig. 7F). As a negative control, we found that knockdown of PCBP1 had no obvious effect on the expression of mutant p53 (R175H) from a reporter vector that carries no sequence from *POLH* 3'UTR (Fig. 7F, Ctrl panel). Interestingly, we found that the level of mutant p53 was decreased by knockdown of PCBP1 for the reporter vectors that carry an intact ARE-B (A11 and ARE-A) (Fig. 7F, A11 and ARE-A panels). In contrast, knockdown of PCBP1 had no effect on mutant p53 expression for reporter vectors that carry ARE-B and ARE-AB region, respectively (Fig. 7F, ARE-B and ARE-AB panels). These data suggest that PCBP1 binds to ARE-B and the poly(rU) nucleotides in ARE-B are crucial for the binding of PCBP1 to *POLH* mRNA.

DISCUSSION

Previous studies have shown that *POLH* expression can be regulated at the transcriptional level by p53 and at post-translational levels by Pirh2 and MDM2 [25, 27, 28]. It is not clear, however, whether *POLH* expression is regulated by other mechanisms. In this study, we found that PCBP1 regulates *POLH* expression via mRNA stability. Thus, an obvious question would be: is there a functional connection between PCBP1 and POLH? Indeed, PCBP1 is known to inhibit tumor invasiveness and metastasis by repressing PRL3 [41] and CD44 [42]. Additionally, dephosphorylated PCBP1 is capable of repressing epithelial-mesenchymal transition (EMT) via decreased translation of disabled-2 (Dab2) and interleukin-like EMT inducer (ILEI) [43]. Consistently, PCBP1 is found to be downregulated in cervical tumor tissues [44], in breast cancer cell lines [45], and during malignant transformation of hydatidiform moles [46]. Since POLH is necessary for the maintenance of genome stability [23, 24], downregulation of PCBP1 in cancer cells would reduce *POLH* mRNA stability, leading to genome instability. Additionally, since POLH plays a role in p53 activation upon DNA damage [25], downregulation of PCBP1 in cancer cells would reduce the level of POLH, thus weakening p53 activation. Thus, further studies are warranted to address the relationship between PCBP1 and POLH in normal and cancer cells, which may provide an insight into the possibility that PCBP1 might be explored as a marker or target for anticancer therapeutic strategies.

PCBP1 is known to recognize poly(rC)- or CU-rich elements in its targets, including tyrosine hydroxylase [47], β -globin [48], androgen receptor [49], collagens [50], erythropoietin [47], and 15-lipoxygenase [51]. PCBP1 also recognizes to non-poly(rC) elements in other targets, including human papilloma virus L2 transcript [52] and Dab2 and ILEI transcripts [43]. However, PCBP1 consensus element has not been defined in these transcripts. Here, we found that PCBP1 regulates *POLH* expression via binding to an AU-rich element located in the proximal *POLH* 3'UTR. Although *POLH* mRNA harbors at least two AREs, our data suggest that only one ARE element is recognized by and responsive to PCBP1. Thus, future studies are needed to define how *POLH* mRNA stability is regulated by PCBP1 and other RNA-binding proteins via the AU-rich element.

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Abbreviations

PCBP1	poly(rC)-binding protein 1
POLH	DNA polymerase eta
ARE	AU-rich element
TLS	translesion DNA synthesis
REMSA	RNA electrophoretic mobility shift assay
UTR	untranslated region
shRNA	short hairpin RNA
GST	glutathione S-transferase

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SUMMARY

DNA polymerase eta (POLH) is mutated in Xeroderma Pigmentosum Variant patients and required for genome stability. However, it is still uncertain how *POLH* expression is regulated at mRNA level. Here we found that *POLH* expression is regulated by poly(rC)-binding protein PCBP1 via mRNA stability.

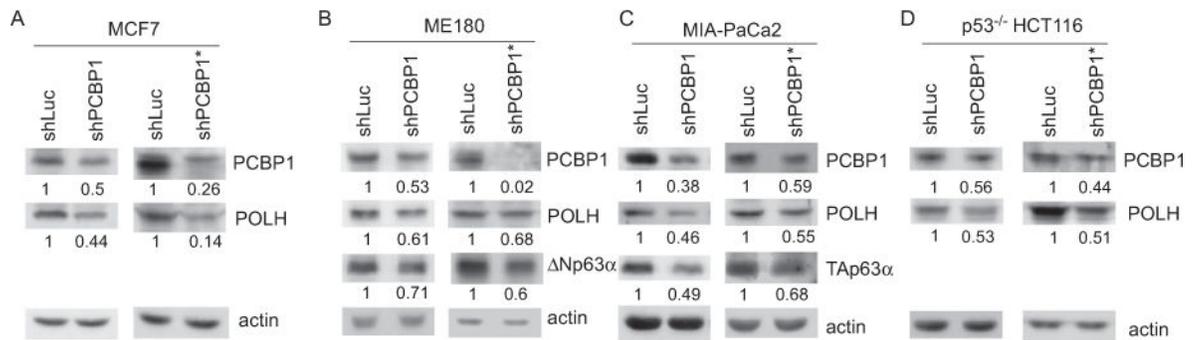


Figure 1. *POLH* expression is decreased upon knockdown of PCBP1

MCF7 (A), ME180 (B), MIA-PaCa2 (C) and p53^{-/-} HCT116 (D) cells were transduced with a lentivirus expressing luciferase shRNA (shLuc) or one of the two PCBP1 shRNAs (shPCBP1 or shPCBP1*), followed by puromycin selection for 3 days. Whole cell lysates were collected and used for western blotting to measure the levels of PCBP1, POLH, actin, Np63α, and TAp63α. The level of actin protein was used as a loading control. Western blots shown in the figure were representative of three independent experiments. The values below the strips were the relative intensities normalized to actin.

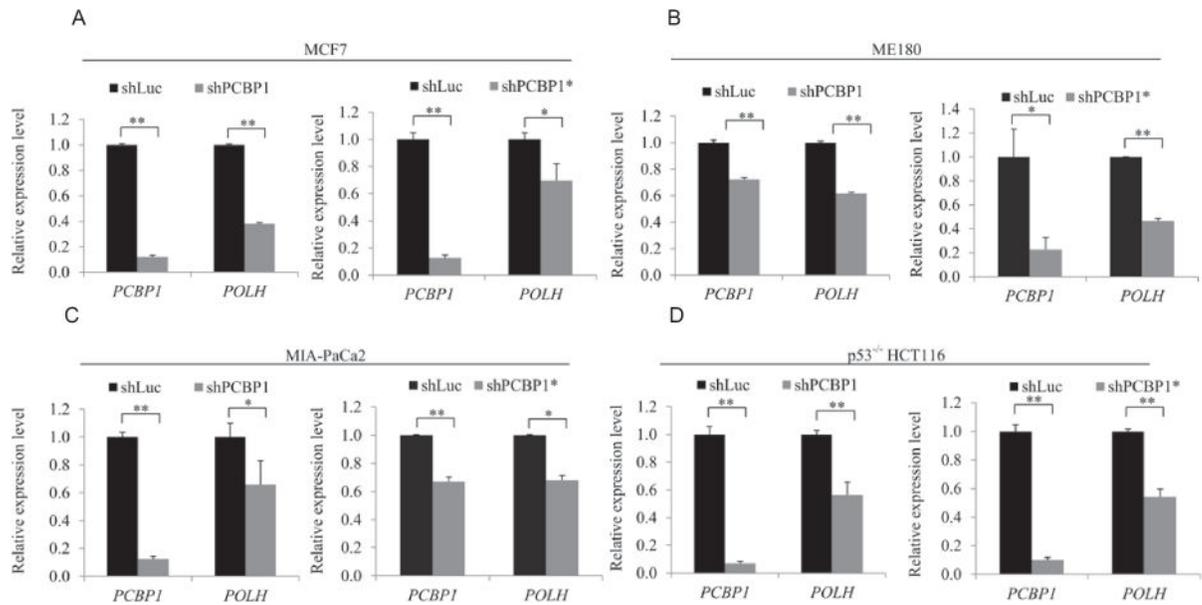


Figure 2. *POLH* mRNA is decreased upon knockdown of *PCBP1*

MCF7 (A), ME180 (B), MIA-PaCa2 (C) and p53^{-/-} HCT116 (D) cells were transduced with a lentivirus expressing a control luciferase shRNA (shLuc) or one of the two *PCBP1* shRNAs (shPCBP1 or shPCBP1*), followed by puromycin selection for 3 days. Total RNA was purified and RT-qPCR was performed to determine the levels of *POLH*, *PCBP1* and actin transcripts. Error bars indicate S.D. and significance was calculated using t-test (**P<0.01, *P<0.05).

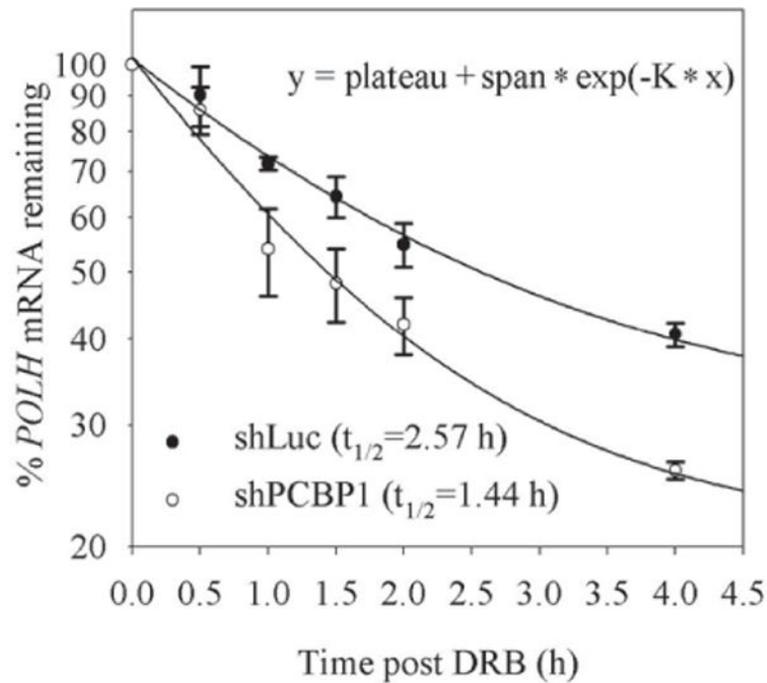


Figure 3. *POLH* mRNA stability is regulated by PCBP1

p53^{-/-} HCT116 cells were transduced with a lentivirus expressing shLuc or shPCBP1 and selected with puromycin for 3 days, followed by treatment with 5, 6-Dichloro-1- β -D-ribofuranosylbenzimidazole (DRB) for the indicated times. The levels of *POLH* and actin transcripts were determined by RT-qPCR. The relative levels of *POLH* transcript were normalized with the levels of actin, which were then plotted along with the times following DRB treatment to determine the relative half-life of *POLH* mRNA. One of the three independent experiments was shown here.

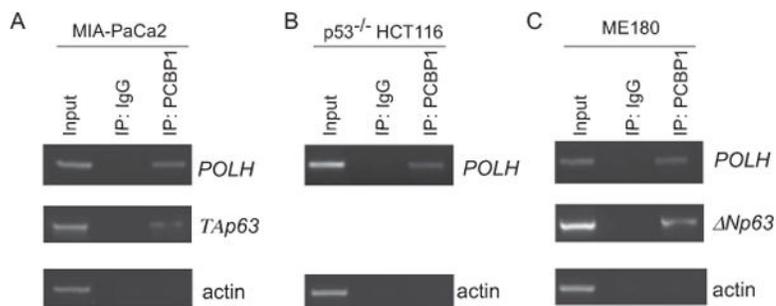


Figure 4. PCBP1 physically interacts with *POLH* transcript *in vivo*

MIA-PaCa2 (A), p53^{-/-} HCT116 (B) and ME180 (C) cells were lysed by immunoprecipitation buffer and incubated with rabbit anti-PCBP1 antibody or control IgG, followed by washing and RNA extraction. RT-PCR was performed to examine the level of *POLH* in the control IgG and anti-PCBP1 immunocomplexes. The levels of *Np63* transcript in ME180 cells, and *TAp63* transcript in MIA-PaCa2 cells, were measured as a positive control. The levels of actin transcript were used as a negative control. The RT-PCR result is representative of at least three independent experiments.

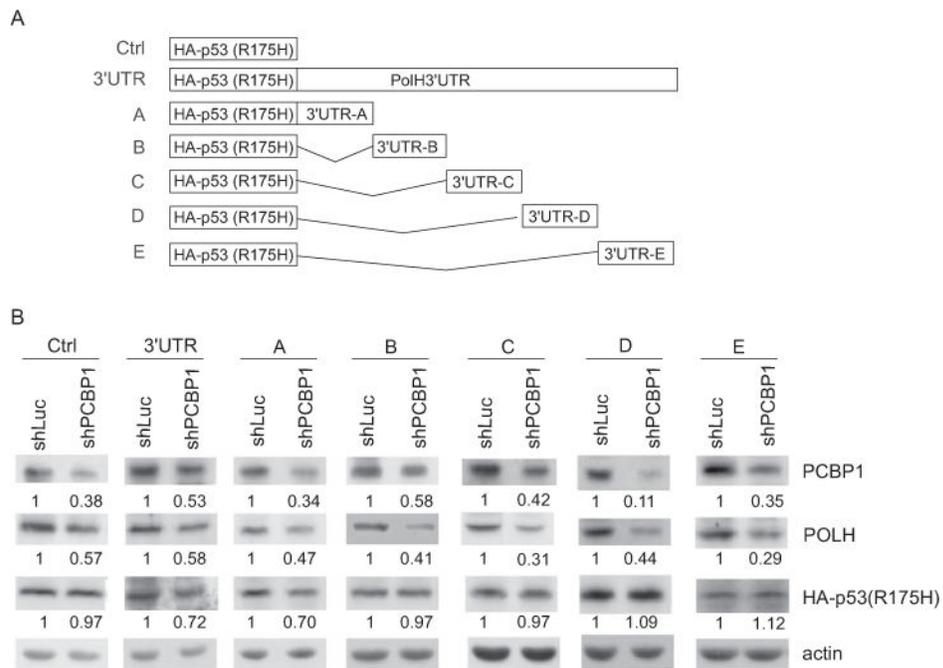


Figure 5. *POLH* 3'UTR is responsive to PCBP1

(A) Schematic presentation of mutant p53 (R175H) reporter vectors that carry none, the full-length, or a part of *POLH* 3'UTR. Ctrl: no *POLH* 3'UTR; 3'UTR: the full-length *POLH* 3'UTR from nt 2447–8412; A: nt 2447–3607 from *POLH* mRNA; B: nt 3515–4619 from *POLH* mRNA; C: nt 4580–5879 from *POLH* mRNA; D: nt 5843–7177 from *POLH* mRNA; E: 7032–8412 from *POLH* mRNA. (B) The reporter vectors were transfected into MIA-PaCa2 cells transduced with a lentivirus expressing luciferase shRNA or PCBP1 shRNA (shPCBP1) for 3 days. Western blots were performed to determine the levels of the reporter (HA-tagged mutant p53 R175H), endogenous *POLH*, PCBP1, and actin. Western blots shown in the figure were representative of three independent experiments. The values below the strips were the relative intensities normalized to actin.

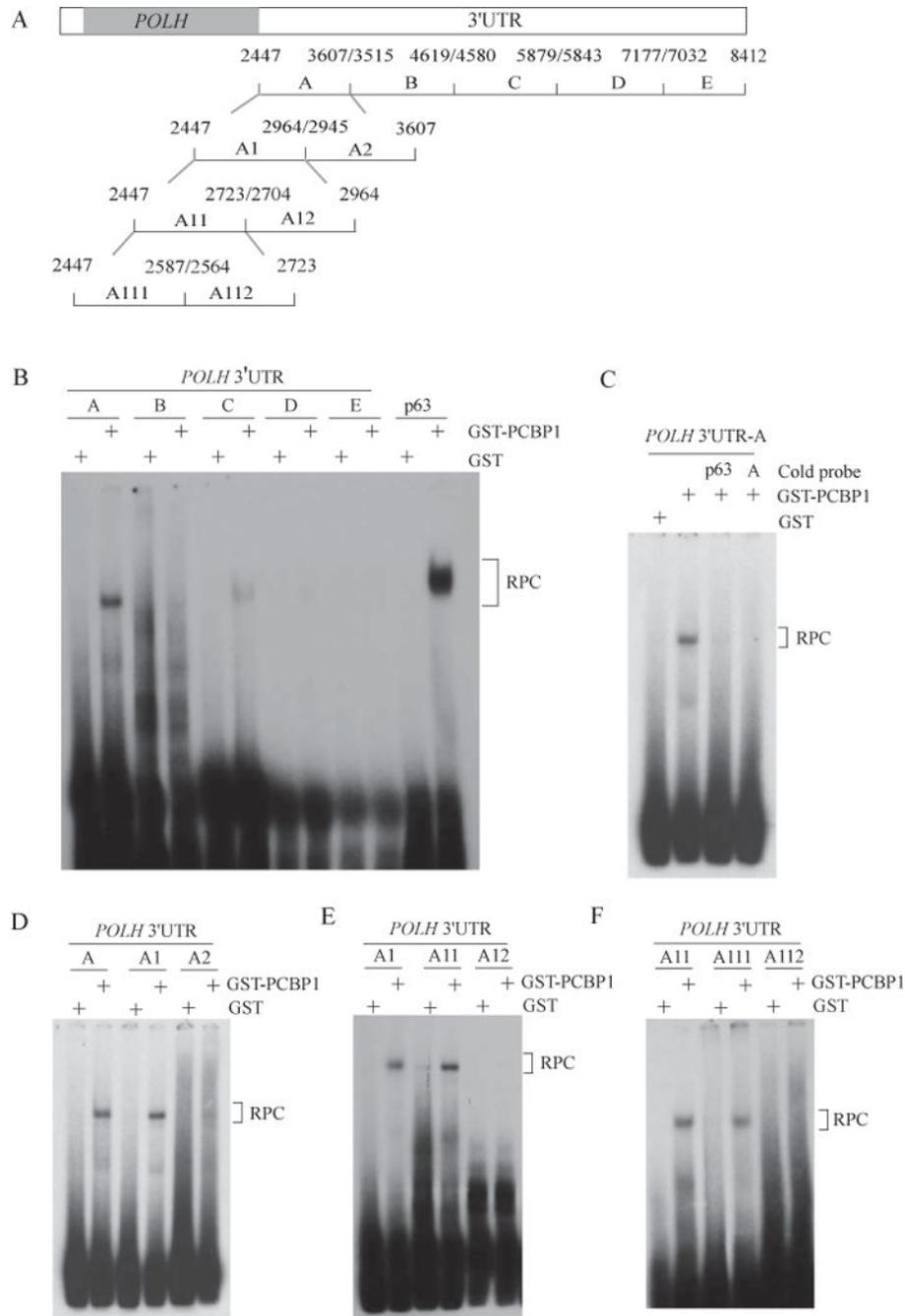


Figure 6. PCBP1 directly binds to *POLH* 3'UTR

(A) Schematic presentation of the transcript of *POLH* and the locations of the probes used for EMSA. (B) PCBP1 binds to fragment A of *POLH* 3'UTR (nt 2447–3607). EMSA was performed with GST or GST-fused PCBP1 along with P^{32} -labeled RNA probes (A, B, C, D, E and p63). p63 probe was used as positive control. RPC, RNA-protein complex. (C) Competition assay was performed by mixing P^{32} -labeled RNA probe A along with or without 50-fold of unlabeled p63 probe or probe A. (D) EMSA was performed by mixing P^{32} -labeled RNA probes (A, A1 and A2) with GST or GST-PCBP1. (E) EMSA was

performed by mixing P³²-labeled RNA probes (A1, A11 and A12) with GST or GST-PCBP1. (F) REMSA was performed by mixing P³²-labeled RNA probes (A11, A111 and A112) with GST or GST-PCBP1. One of the two independent experiments was shown here.

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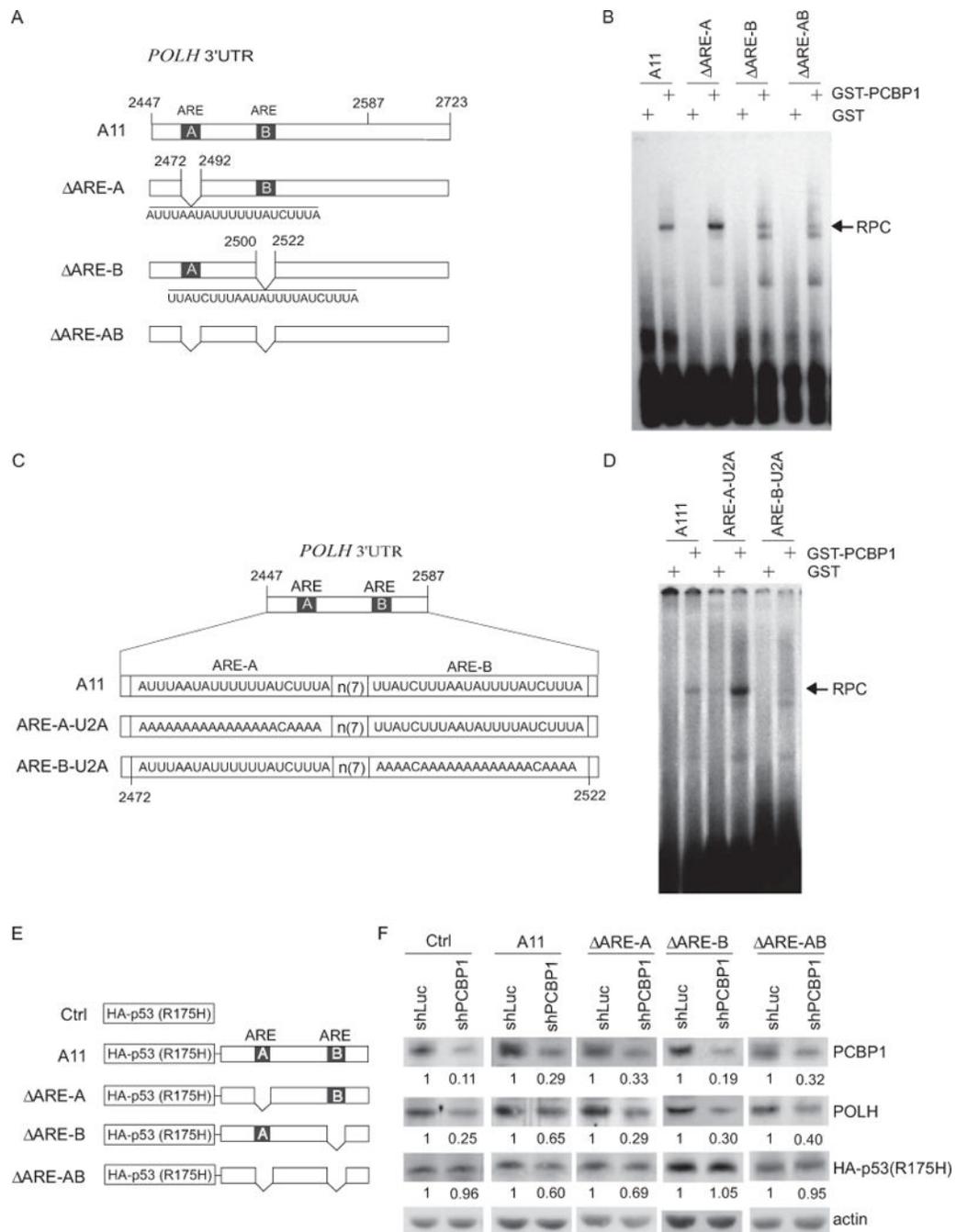


Figure 7. An ARE element in *POLH* 3'UTR is recognized by and responsive to PCBP1
(A) Schematic presentation of the wild-type and deletion mutant probes used for REMSA.
(B) REMSA was performed by mixing P³²-labeled probes (A11, ARE-A, ARE-B and ARE-AB) with GST or GST-PCBP1 to identify the PCBP1-binding site in *POLH* 3'UTR. RPC, RNA-protein complex. **(C)** Schematic presentation of wild-type and mutant probes used for REMSA. **(D)** Poly(rU) nucleotides in ARE-B of *POLH* 3'UTR are crucial for PCBP1 binding. REMSA was performed by mixing P³²-labeled RNA probes (A111, ARE-A-U2A and ARE-B-U2A) with GST and GST-PCBP1. One of the two independent

experiments was shown here. **(E)** Schematic presentation of the reporter vectors for identification of the PCBP1-binding site in *POLH* 3'UTR. **(F)** ARE-B in *POLH* 3'UTR is responsive to PCBP1. The reporter vectors were transfected into MIA-PaCa2 cells transduced with a lentivirus expressing luciferase shRNA or PCBP1 shRNA (shPCBP1) for 3 days. The levels of the reporter (HA-tagged mutant p53 R175H), endogenous *POLH*, PCBP1, and actin were determined by western blotting. Western blots shown here were representative of three independent experiments. The values below the strips were the relative intensities normalized to actin.

Table 1

Primers used in this study.

Primer name	Sequence
Primers for RT-PCR	
POLH-exo4-F	5'-tcgagccattgaataagcc-3'
POLH-exo5-R	5'-acaaggtcagcctatctcgg-3'
actin-exo3-F	5'-ctgaagtacccatcagcagcgca-3'
actin-exo4-R	5'-ggatagcacagcctggatagcaacg-3'
PCBP1-F	5'-ggcgggtgaatcaaaaga-3'
PCBP1-R	5'-gagcggagaaatggtgtt-3'
p63-1751-F	5'-gaggttggctgttcatcat-3'
p63-2023-R	5'-gtgaatcgcacagcatcaat-3'
Np63-153-F	5'-ggaaaacaatcccagactc-3'
Np63-513-R	5'-tgggtcatcaccttgatct-3'
Primers for generation of reporter vectors	
POLH-XhoI-2447-F	5'-atcgctcagtgctgccctcaggcttgcctgtaggatta-3'
POLH-NheI-8412-R	5'-atcgctcagctattgtacagaataaaaatgtttattgaatac-3'
POLH-NheI-3607-R	5'-atcgctcagcctgacgacagaggaga-3'
POLH-XhoI-3515-F	5'-atgcctcagaatgtaatgagacttgcatagtt-3'
POLH-NheI-4619-R	5'-atcgctcagctgccctagttaccatatacactt-3'
POLH-XhoI-4580-F	5'-atcgctcaggaagccttgaaccctaaa-3'
POLH-NheI-5879-R	5'-atcgctcagccacctggtcattatcttttag-3'
POLH-XhoI-5843-F	5'-atcgctcagggagaaatgctgatctaaaaga-3'
POLH-NheI-7177-R	5'-atcgctcagcattcaggtgatcctccc-3'
POLH-XhoI-7032-F	5'-atgcctcagggaggtgggtgactactgga-3'
POLH-NheI-2723-R	5'-atcgctcagccaagcccacacactttta-3'
POLH-XhoI2447-2471-	5'-atcgctcagtgctgccctcaggcttgccttagg-3'
Primers for generation of REMSA RNA probes	
POLH-2447-T7-F	5'-ggatcctaatacagactcactatagggagtgctgccctcaggcttgcctg-3'
POLH-3607-R	5'-cctgacgacagaggaga-3'
POLH-3515-T7-F	5'-ggatcctaatacagactcactatagggagaaatgtaatgagacttgcatagtt-3'
POLH-4619-R	5'-tgcctagttaccatatacactt-3'
POLH-4580-T7-F	5'-ggatcctaatacagactcactatagggaggaagccttgaaccctaaa-3'
POLH-5879-R	5'-cacctggtcattatcttttag-3'
POLH-5843-T7-F	5'-ggatcctaatacagactcactatagggaggaagaaatgctgatctaaaaga-3'
POLH-7177-R	5'-gattcaggtgatcctccc-3'
POLH-7032-T7-F	5'-ggatcctaatacagactcactatagggagaggtgggtgactactgga-3'
POLH-8412-R	5'-tattgtacagaataaaaatgtt-3'
POLH-2964-R	5'-ggctgtctcaaacctga-3'
POLH-2945-T7-F	5'-ggatcctaatacagactcactatagggagtcaggagtttgagaccagcc-3'
POLH-2723-R	5'-caagcccacacactttta-3'
POLH-2704-T7-F	5'-ggatcctaatacagactcactatagggagtaaaaagtgtgtggccttg-3'

Primer name	Sequence
POLH-2587-R	5'-tcagcacctaatggattat-3'
POLH-2564-T7-F	5'-ggatcctaatacgcactactataggagagaaaaataccattaggtgctga-3'
T7-deltaARE-A-F	5'-ggatcctaatacgcactactataggagtgctgccctcaggctgcctgtaggcagatctttatcttaaat-3'
T7-deltaARE-B-F	5'-ggatcctaatacgcactactataggagtgctgccctcaggctgcctgtaggat-3'
T7-deltaARE-AB-F	5'-ggatcctaatacgcactactataggagtgctgccctcaggctgcctgtaggcagatctcagattccctgagaaaggaat-3'
POLH-ARE-A-t2a-F	5'-tgctccctcaggctgcctgtaggagaaaaaaacaaaacagatctttatcttaaat-3'
POLH-ARE-B-t2a-F	5'-tgctccctcaggctgcctgtaggat-3'
T7-POLH-2447-2467-F	5'-ggatcctaatacgcactactataggagtgctgccctcaggctgcctg-3'

F, forward; R, reverse.