CRL4\textsuperscript{Wdr70} regulates H2B monoubiquitination and facilitates Exo1-dependent resection

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Double-strand breaks repaired by homologous recombination (HR) are first resected to form single-stranded DNA, which binds replication protein A (RPA). RPA attracts mediators that load the Rad51 filament to promote strand invasion, the defining feature of HR. How the resection machinery navigates nucleosome-packaged DNA is poorly understood. Here we report that in \textit{Schizosaccharomyces pombe} a conserved DDB1-CUL4-associated factor (DCAF), Wdr70, is recruited to DSBs as part of the Cullin4-DDB1 ubiquitin ligase (CRL4\textsuperscript{Wdr70}) and stimulates distal H2B lysine 119 mono-ubiquitination (uH2B). Wdr70 deletion, or uH2B loss, results in increased loading of the checkpoint adaptor and resection inhibitor Crb2\textsuperscript{53BP1}, decreased Exo1 association and delayed resection. Wdr70 is dispensable for resection upon Crb2\textsuperscript{53BP1} loss, or when the Set9 methyltransferase that creates docking sites for Crb2 is deleted. Finally, we establish that this histone regulatory cascade similarly controls DSB resection in human cells.
DNA damage, if not accurately repaired, poses a threat to genome integrity. When incorrectly repaired, a single DNA double-strand break (DSB) can result in loss of genetic information and gross chromosomal rearrangement, both signatures of carcinogenesis. In metazoans, DSBs are mainly repaired by non-homologous end joining (NHEJ) or homologous recombination (HR). NHEJ requires minimal DNA end processing and is innately error prone because chemically damaged or lost bases are not replaced. In contrast, HR requires extensive 5′-to-3′ DNA end resection. This generates a stretch of RPA-coated single-stranded DNA (ssDNA) that attracts repair factors to build a Rad51 filament to initiate a homologous search. Once a homologous template is found, repair is accurate because DNA synthesis can replace bases that were either lost or chemically modified at the original damage site.

Resection is initiated by an Mre11–Rad50–Nbs1 complex (MRN) and the associated C-terminal-binding protein interacting protein (ChIP) repair factor. DNA is nicked by the Mre11 nuclease on the strand with the 5′ end, ~15–20 bases 3′ from the break. In the yeast model system, this Mre11 activity is regulated by the ChIP homologue. The small region from the nick site to the break is subsequently degraded 3′ to 5′ back to the break, releasing DSB-bound proteins such as the NHEJ initiator Ku. MRN- and ChIP-dependent ‘clipping’ provides an entry point for the 5′ to 3′ resection factors. Resection depends on either Exo1 or Dna2 (which function in complex with BLM: known as Sgs1 or Rqh1 in S. cerevisiae and S. pombe, respectively). Once resection is initiated, repair of the DSB is channelled into HR pathways such as sister chromatid repair or single-strand annealing. Thus, the regulation of resection initiation and its subsequent progression are thought to determine which repair pathway, HR, NHEJ or microhomology-mediated end joining, acts to repair the break.

DSB repair and its regulation occur on chromatin. A cascade of proteins associate with chromatin in proximity to a DSB and this is largely dependent on H2AX phosphorylation by DNA damage signalling proteins. This accumulation of proteins into foci has been suggested to serve a number of specific functions; increasing the local concentration of repair factors, allowing amplification of DNA damage signals and facilitating the regulation of resection to promote the use of the appropriate DNA repair pathway. In addition to H2AX phosphorylation, multiple other histone modifications have been directly linked to DSB signalling and repair in both yeast and human cells, but many details regarding how chromatin modification coordinates with the signalling, processing and repair of DNA damage remain elusive. The Cullin 4 ring E3 ubiquitin ligases (CRL4s) regulate a wide variety of biological processes including chromatin remodelling, DNA replication and repair. Like SCF (Skp1-CUL1-F box) complexes, CRL4s consist of a core set of components (CUL4-DDB1-ROC) associated with one of a variety of substrate-specific adapters that target defined proteins to CRL4 for ubiquitin-dependent modification. These adaptors form a family of ‘DWD’ (DDB1-binding WD40) proteins that define multiple CRL4 complexes (that is, CRL4Cdt2; CRL4Ddb2; and so on). Database search and biochemical analysis predict that about one-third of the human WD40 proteins contain DWD motif and thus are prospective CRL4 targeting subunits. Only few of these have been biologically characterized.

Fission yeast CRL4Cdt2 was shown to regulate dNTP synthesis during DNA replication and repair by promoting degradation of Spd1, a ribonucleotide reductase (RNR) inhibitor. Deletion of spd1 partially alleviates the hypersensitivity of cul4Δ and ddb1Δ mutants to genotoxic agents, but does not fully reverse these phenotypes. This indicated that CRL4s regulate the DNA damage response via additional substrate-targeting subunits.

Based on this hypothesis, we set out to identify additional roles for CRL4s in the DNA damage response. We identified Wdr70, a previously uncharacterized WD40-repeat protein. We demonstrate that Wdr70 associates with Ddb1 to form a novel CRL4 complex and that the level of this complex is increased in response to DNA damage. We establish that CRL4Wdr70 specifically promotes resection of DSBs and HR by regulating the monoubiquitination status of histone H2B (uH2B). We show that this mechanism is evolutionarily conserved in human cells, identifying a novel aspect of chromatin regulation during DSB repair.

Results
Wdr70 forms a damage-induced CRL4 complex. Fission yeast CRL4Cdt2 regulates dNTP synthesis during DNA replication and repair by promoting degradation of Spd1, a RNR inhibitor. To identify additional roles for CRL4s in the DNA damage response, we introduced a cDNA library into ddb1Δ spd1Δ cells and screened for altered genotoxin sensitivity. We identified wdr70Δ (SPAC343.17c), expression of which increased sensitivity to a range of treatments (see Supplementary Fig. 1a for details). Wdr70 is a conserved nuclear WD40-repeat protein with a ‘DWD’ motif, interacts with DCAF and the CRL4 subunit, Ddb1 (ref. 14). We showed that Wdr70 physically interacts with Ddb1 (Fig. 1b) in a DWD-dependent manner (Supplementary Fig. 1d), that this interaction is resistant to ethidium bromide (Supplementary Fig. 1e) and enhanced upon ultraviolet light treatment or exposure to ionising radiation (IR) (Fig. 1c). This indicates the formation of a CRL4Wdr70 complex that is DNA damage stimulated.

wdr70 null cells (wdr70A), or cells harbouring a mutation in the DWD motif that prevents binding to Ddb1 (wdr70-wd) showed prolonged Chk1 phosphorylation and slow growth and an elongated cell phenotype typical of delayed G2/M progression (Supplementary Fig. 1g). These phenotypes are reminiscent of S. pombe DNA repair mutants that accumulate unrepaired spontaneous DNA damage. Consistent with increased endogenous lesions, wdr70Δ viability was dependent on rad3Δ, a core DNA structure checkpoint gene, and untreated wdr70Δ cells showed low plating efficiency, a high incidence of mitotic catastrophe and elevated spontaneous Rad52 foci (Supplementary Fig. 2a–d). When compared with wdr70Δ controls, wdr70Δ cells displayed viability loss upon ultraviolet or methyl methanesulfonate (MMS) treatment (Fig. 1d). Although not sensitive to IR and CPT treatment (Supplementary Fig. 2e), wdr70Δ cells showed prolonged Chk1 phosphorylation and enhanced mitotic delay in response to treatment with both IR and MMS (Supplementary Fig. 2f). Repair capacity, as judged by recovery of full-length chromosomes upon pulse field gel electrophoresis, was reduced in response to MMS treatment, but not IR treatment (Fig. 1e).

CRL4Cdt2 degrades Spd1 to regulate RNR to promote nucleotide synthesis for postsynaptic gap filling and efficient HR. We observed that Spd1 levels are increased in wdr70Δ G2 cells (spd1 mRNA levels are elevated) and that, consistent with this, concomitant spd1 deletion partially rescued wdr70Δ damage sensitivity (Supplementary Fig. 2g). To ensure the Wdr70-dependent DNA damage response phenotypes that we characterize below were independent of Spd1-mediated dNTP regulation, we performed all further genetic analysis in spd1Δ backgrounds. Unless otherwise stated as spd1Δ, all strains labelled as ‘WT’ are spd1Δ.

Wdr70 promotes long-range resection at DSB. The mechanism of spontaneous DNA break repair can be assessed in S. pombe
and Wdr70. MYC, TAP and HA-tagged proteins were expressed from their genomic loci. (the indicated time points after MMS or IR treatment. (hypersensitive control. n
¼ repeats. HR, homologous recombination. Error bars
gene conversion or a recombination event that deletes the
exo1 proximal and distal to the break. In contrast, in either
wdr70 cells: extensive foci
remained in
wdr70 control cells 10 h after treatment, whereas foci in
proximal (0.2 kb) and distal (3 kb) to an induced DSB site
Both Wdr70 and Ddb1 were found to directly associate both
requirement for the Signalosome subunits Csn1 and Csn5 in
recruits CRL4 Wdr70. As the COP9-Signalosome is a known
Wdr70 mediates uH2B spreading by RNF20-RNF40 (HULCRhp6).
with DNA proximal (0.2 kb) to the DSB with similar kinetics for
both wdr70? and wdr70? cells but delayed association was
evident distal (3 and 9.4 kb) to the break in wdr70? (Fig. 2c).
mre11? cells were, as expected, partially defective in both
proximal and distal Rpa1 association.

Wdr70 mediates uH2B spreading by RNF20-RNF40 (HULCRhp6).
To determine if the observed defect in resection was due to a
direct role for Wdr70 or was an indirect consequence of other
changes to cell metabolism, we used ChIP analysis to establish if
CRL4Wdr70 subunits were recruited to sites of DNA breakage.
Both Wdr70 and Ddb1 were found to directly associate both
proximal (0.2 kb) and distal (3 kb) to an induced DSB site
(Fig. 2d,e and Supplementary Fig. 3c,d). Loss of Ddb1 association
in the wdr70? background could not be complemented by
wdr70-wd mutant expression (Fig. 2d), indicating that Wdr70
recruits CRL4Wdr70. As the COP9-Signalosome is a known
regulator of CRL4 ubiquitin ligase activity, we tested the
requirement for the Signalosome subunits Csn1 and Csn5 in
wdr70?-mediated resection. Both the csn1? and csn5? mutations
reduced the amount of ssDNA present distal to the induced
DSB, indicating a resection defect (Supplementary Fig. 3e). These
phenotypes were not additive with wdr70? when the mutations
were combined in the same cells, consistent with the
Wdr70-dependent regulation of resection being mediated by
ubiquitin ligation.

Because CRL4s have previously been linked to the regulation
of histone dynamics and chromatin status is known to
influence DSB processing, we systematically screened known
using a heterallelic recombination assay in which non-tandem
direct repeats of ade6 heteroalleles can revert to ade+ by either
gene conversion or a recombination event that deletes the
intervening sequence. When compared with wdr70? controls,
sporontic HR-dependent gene conversion and deletion was
suppressed in a wdr70? background (Fig. 1f and Supplementary
Fig. 2h). Consistent with this, in a plasmid re-joining assay
outcomes associated with NHEJ were elevated 3.7-fold (Fig. 1g).
This indicated that Wdr70 functions to promote HR repair of
DSBs and, in its absence, NHEJ becomes more central. We next
characterized the kinetics of HR by monitoring IR-induced Rpa1
and Rad52 foci. Peak levels of Rpa1 and Rad52 foci were delayed in
wdr70? when compared with wdr70? cells: extensive foci
remained in wdr70? cells 10 h after treatment, whereas foci in
wdr70? cells were resolved by 4 h (Fig. 2a and Supplementary
Fig. 3a,b). This suggested that resection was delayed in the
wdr70? background. To explore this further, we induced a single
DSB using the HO endonuclease and monitored resection by
PCR. Resection results in ssDNA that is refractory to restriction
endonuclease digestion. Thus, following digestion, resected DNA
remains intact for amplification. Two Apol sites (Fig. 2b) were
assayed, one immediately proximal (35 bp) to the HO-induced
DSB site, the other distal (3 kb) from the break.

In wdr70? cells, DSB resection was apparent both immediately
proximal and distal to the break. In contrast, in either wdr70? or
exo1? control cells, resection 3 kb from the break was impaired
(Fig. 2b). In this semi-quantitative assay, mre11? cells displayed a
reduction in both proximal and distal resection, as expected.
To confirm that Wdr70 affected long-range resection, we performed
Rpa1 chromatin immune-precipitation (ChIP). Rpa1 associated
Figure 1 | CRL4Wdr70 influences DSB repair pathway selection. (a) The putative DWD motif of Wdr70. (b) Co-immunoprecipitation (co-IP) of tagged Ddb1 and Wdr70. MYC, TAP and HA-tagged proteins were expressed from their genomic loci. (c) Co-IP as in b following ultraviolet (UV; 500 J m−2) or ionising radiation (IR; 80 Gy) treatment. (d) Clongenic survival analysis of wdr70? and wdr70?Δ cells following MMS or UV exposure. rad3? was used as a
hypersensitive control. n ¼ 3 biological repeats. Error bars ¼ s.d. (e) Pulse field gel electrophoresis of genomic DNA extracted from wdr70? and wdr70?Δ cells at the
indicated time points after MMS or IR treatment. (f) Comparative analysis of HR efficiency between wdr70? and wdr70?Δ cells (spd1? background) using a
heterallelic recombination assay. Ratios represents the proportion of conversion (ade+ his+) to deletion (ade− his−) type repair products. n ¼ 3 biological
repeats. HR, homologous recombination. Error bars ¼ s.d. (g) Comparative analysis of NHEJ efficiency using a plasmid re-joining assay (spd1? background).
n ¼ 3 biological repeats. NHEJ, non-homologous end joining. Error bars ¼ s.d.
histone modifications in IR-treated wdr70+ and wdr70Δ cell lysates (Supplementary Fig. 4a,b). The major variation we observed was H2B lysine 119 mono-ubiquitination and we found that induction of H2B by DNA damage was partially dependent on wdr70 or ddb1 (Fig. 3a). H2B monoubiquitination is catalysed by the RNF20–RNF40–UbcH6 complex23 (known as HULCRhp6 in S. pombe24) and promotes chromatin remodelling during transcription by influencing H3 methylation25. RNF20-dependent H2B monoubiquitination has also been linked to the promotion of DSB repair through the regulation of resection and the recruitment of HR factors in human cells26,27.

Consistent with H2B monoubiquitination functioning at DNA repair sites, we found that H2B in S. pombe was enriched both proximal (0.2 kb) and distal (3 and 9.4 kb) to an induced DSB with kinetics similar to Rpa1 (Fig. 3b). To explore the role of CRL4Wdr70 in the regulation of H2B at DNA damage sites, we examined H2B induction in the wdr70 mutant backgrounds. Either deletion of wdr70 (Fig. 3b) or a mutation of the DWD motif (Supplementary Fig. 4c) reduced H2B enrichment at sequences distal to an induced DSB but not at the proximal sequences. As rhp6 is required for H2B enrichment (Supplementary Fig. 4d), we propose that CRL4Wdr70 is not a ligase for H2B-K119 ubiquitination, but regulates H2B ubiquitination by HULCRhp6.

If the resection defect observed in wdr70Δ cells is caused by failure to regulate H2B, then single-stranded DNA production around an induced DSB should be similarly affected in rhp6Δ cells (the E2 for HULCRhp6)24 as well as in cells harbouring H2B-K119R mutations. Both rhp6Δ and H2B-K119R cells displayed attenuated ssDNA at DSB-distal sites (Fig. 3c). We also observed that the double H2B-K119R wdr70Δ mutant cells demonstrated epistasis for genotoxic treatments (Supplementary Fig. 4e), consistent with CRL4Wdr70 regulating H2B monoubiquitination by HULCRhp6. Further support for CRL4Wdr70 regulating H2B, but not directly modifying H2B itself, comes from the observation that concomitant deletion of wdr70 and ubp8 (an H2B-specific de-ubiquitinase28) partially rescued the wdr70Δ-dependent defects in H2B and MMS survival (Supplementary Fig. 4f,g), whereas ubp8 deletion did not rescue rhp6Δ sensitivity (Supplementary Fig. 4g). Using RNA sequencing profiling of genome-wide gene expression (Supplementary Table 1), we did not observe changes to the expression of genes implicated in resection. Similarly, assessing the protein levels of a variety of repair factors (Supplementary Fig. 4h) did not identify significant changes. Taken together, our data thus suggest that CRL4Wdr70 functions to regulate Rhp6-dependent H2B ubiquitination surrounding DSBs in order to facilitate long-range resection.

To further explore the role of Wdr70 in establishing H2B chromatin domains at DSB sites, we monitored the recruitment of the HULCRhp6 complex subunits. Rhp6 E2 ligase recruitment was evident both proximal (0.2 kb) and distal (3 kb) in wdr70+ cells, whereas distal but not proximal recruitment was reduced in wdr70Δ cells (Fig. 3d). A decrease in recruitment of Br1l1, the HULCRhp6 complex E3, was also observed at the distal, but not the proximal location (Supplementary Fig. 4i). Interestingly, the chromatin loading of Wdr70 was decreased both break-proximal and distal in the H2B-K119R background (Fig. 3e), indicating that initial H2B monoubiquitination is required to recruit CRL4Wdr70 to facilitate the subsequent modification cascade. Consistent with this interpretation, Wdr70 and H2B could be co-immunoprecipitated from wild type cell extracts in the presence of ethidium bromide, but not the H2B-K119R cell extracts (Fig. 3f).

Resection is initiated by MRN- and Ctp1-dependent nuclease activity to generate a short ssDNA substrate. This is followed by further resection dependent on Exo1 and Rqh1BLM.Dna2.
Our data suggest the initial short tract of ssDNA triggers proximal uH2B, with further uH2B spreading dependent on CRL4\(^{Wdr70}\). In support of this recruitment of both Wdr70 and Rhp6 plus the uH2B modification were significantly decreased both proximal and distal in ctp1Δ cells (Fig. 3g). Since neither CRL4\(^{Wdr70}\) nor uH2B influence proximal resection (Fig. 3c), we conclude that HULC\(^{Rhp6}\) is recruited to the initial ssDNA-RPA exposed proximal to the DSB to form a core uH2B domain. Consistent with this, we were able to co-immunoprecipitate Rhp6 and Rpa1 in the presence of ethidium bromide (Fig. 3h). Thus, our data indicate that uH2B is modified in response to a DSB in two separable stages: an initial core domain is triggered by short-range resection followed by a subsequent CRL4\(^{Wdr70}\)-dependent spreading distal to the DSB (Fig. 4a).

**DSB recruitment of Exo1 nuclease by Wdr70 and uH2B.** To distinguish which resection pathway Wdr70 influences, we monitored recruitment of Mre11, Ctp1, Exo1 and Rqh1\(^{BLM}\) both proximal and distal to an induced DSB (Fig. 4b). As expected, Mre11 and Ctp1 were recruited to proximal, but not distal, sites. Their profiles were not affected by loss of CRL4\(^{Wdr70}\). Rqh1\(^{BLM}\), which participates with Dna2 in long-range resection, was recruited at both proximal and distal sites and was unaffected by loss of CRL4\(^{Wdr70}\). This implies that Rqh1-dependent resection is independent of uH2B. In contrast, Exo1 recruitment at both the proximal and the distal loci was severely impaired by CRL4\(^{Wdr70}\) loss. This implies that Wdr70, and thus uH2B, mainly influences Exo1-dependent resection. Consistent with this, we observed that \(rqp1\Delta\) wdr70Δ double mutants, displayed additive resection defects (Fig. 4c). Furthermore, \(rqp1\Delta\) wdr70Δ, but not exo1Δ wdr70Δ, showed synergistic MMS sensitivity (Supplementary Fig. 5a) and concomitant deletion of exo1 did not exacerbate the wdr70Δ HR deficiency as assayed by spontaneous heteroallelic recombination (Fig. 4d).

*In vitro*, resection by Exo1, as opposed to resection by the Rqh1\(^{BLM}\)-Dna2 complex, is blocked by nucleosomes and removal of H2A-H2B dimers partially overcomes this\(^{29}\). We therefore tested if the decreased recruitment of Exo1 observed in wdr70Δ cells was epistatic to the loss of uH2B. In cells where the wdr70Δ and H2B-K119R mutants were combined, no statistically significant changes were seen for Exo1 recruitment when compared with either the wdr70Δ or H2B-K119R backgrounds (Fig. 4e). Overexpression of Exo1 did not rescue the wdr70Δ resection defect indicating that uH2B promotes Exo1 resection activity, rather than its initial recruitment (Supplementary Fig 5b). We next quantified the recruitment of Wdr70 and H2B monoubiquitination in exo1Δ, rqp1Δ and exo1Δ rqp1Δ double mutant cells, the latter of which are predominantly defective in long-range resection (Supplementary Fig 5c). In contrast to control and single mutant cells, the exo1Δ rqp1Δ double mutant exhibited compromised Wdr70 recruitment and H2B modification distal to a DSB. This is distinct from the observation that DSB proximal uH2B is independent of long-range resection (but is compromised in ctp1Δ cells, where the initial resection is defective). We conclude that Wdr70-dependent uH2B spreading from a DSB is co-operative with ongoing resection to allow access of the Exo1-dependent resection machinery.
Catalysed uH2B core proximal to the DSB. (wdr70 and 3 kb from the DSB following induction in the DSB in immunoprecipitation (ChIP) in induced DSB in repeats. Error bars ¼ PCR across the DSB induction. CRL4Wdr70 facilitates the loading of HULCrp6 and the spreading of uH2B distal from the break site after formation of the HULCrp6-53BP1 and its homologues (Crb253BP1 in S. cerevisiae). Resection is regulated by multi-layered mechanisms, of which Wdr70 counteracts the Crb253BP1-dependent resection barrier.

**Figure 4 | CRL4Wdr70 promotes recruitment of Exo1 to DSB. All genetic backgrounds are set9Δ.** (a) A two-step model for H2B mono-ubiquitination after DSB induction. CRL4Wdr70 facilitates the loading of HULCrp6 and the spreading of uH2B distal from the break site after formation of the HULCrp6, catalysed uH2B core proximal to the DSB. (b) An experiment showing the recruitment of Exo1, Rqh1, Ctp1 and Mre11 when assayed by chromatin immunoprecipitation (ChIP) in induced DSB in repeats. Error bars ¼ s.d. **P<0.01 (t-test). (c) Heteroallelic recombination assay for wdr70Δ, exo1Δ and the double wdr70Δ exo1Δ background. n = 3 biological repeats. Error bars ¼ s.d. **P<0.01 (t-test). NS, no statistical significance between strains. (e) Exo1 enrichment measured by ChIP 0.2 and 3 kb from an induced DSB in wdr70Δ, H2B-K119R and wdr70Δ H2B-K119R backgrounds to assess epistasis.

**Figure 5 | CRL4Wdr70 suppresses Crb253BP1 loading at DSBs. All genetic backgrounds are set9Δ.** (a) Recruitment of Crb253BP1 assayed by ChIP at 0.2 and 3 kb from the DSB following induction in wdr70Δ+ (WT) and wdr70Δ backgrounds. (b) Recruitment of Crb253BP1 assayed by ChIP at 0.2 and 3 kb from the DSB in set9Δ, wdr70Δ and set9Δ wdr70Δ double mutant backgrounds. (c) ChIP assay of H4K20me2 enrichment 0.2 and 3 kb from the DSB in wdr70Δ, wdr70Δ and H2B-K119R backgrounds. (d) Recruitment of Exo1 assayed by ChIP (left) and resection analysed by quantitative PCR (right) 3 kb distal to an HO break in the crb2-F400A, wdr70Δ and crb2-F400A wdr70Δ double mutant backgrounds. (e) An equivalent experiment as d for set9Δ, wdr70Δ and set9Δ wdr70Δ double mutant backgrounds. All panels: n = 3 biological repeats. Error bars ¼ s.d. **P<0.01, **P<0.01 (t-test).

Wdr70 counteracts the Crb253BP1-dependent resection barrier. Resection is regulated by multi-layered mechanisms, of which 53BP1 and its homologues (Crb253BP1 in S. pombe and Rad53BP1 in S. cerevisiae) are the most studied. In S. pombe, Crb253BP1 associates with chromatin through co-operative binding to γH2A and di-methylated H4K20 (H4K20me2) via its BRCT and Tudor domains, respectively. All H4K20me2 is dependent on the Set9 methyltransferase in fission yeast.

In the wdr70Δ mutant, we observed that both proximal and distal DSB recruitment of Crb253BP1 was increased when compared with wdr70Δ controls (Fig. 5a). This increase was lost upon concomitant deletion of the Set9 (Fig. 5b). Consistent with Crb253BP1 being recruited via H4K20me2, the level of H4K20me2 was elevated in both wdr70Δ and K119R mutants both proximal and distal to the DSB (Fig. 5c and Supplementary Fig. 5d). This did not correlate with a global increase in H4K20me2...
Wdr70 promotes resection in human cells. As with yeasts, metazoan 53BP1 is recruited to DSBs where it inhibits resection and promotes NHEJ35–37. We assessed the conservation of the CRL4 WDR70 DNA repair function in human cells by depletion of either DDB1 or WDR70 using short interfering RNA (siRNA) on HEK293T cells. Both DDB1 and WDR70 depletion impaired resection, as judged by the attenuation of ionizing irradiation-induced foci for Rad51, RPA32 and RPA32-S33 phosphorylation (Fig. 6a,b and Supplementary Fig 6a–d). Cells deficient for either DDB1 or WDR70 were also sensitive to treatment with ultraviolet, CPT or IR (Supplementary Fig. 6e). We next examined the induced levels of uH2B. The increase of total uH2B that is observed in response to treatment with genotoxic agents was attenuated upon CPT treatment of cells in which CUL4A, DDB1 or WDR70 were depleted by siRNA treatment when compared with a control cells treated with scrambled siRNA. A similar trend was evident after IR treatment for cells depleted of DDB1 or WDR70 (Fig. 6c). This suggests that, in human cells as in S. pombe, CRL4 Wdr70 participates DNA damage-dependent uH2B and the regulation of resection.

Using an I-SceI-mediated DSB system38, we found that, when WDR70 was inactivated by CRISPR technology (Fig. 6d), NHEJ usage increased and HR and SSA were compromised (Fig. 6e). In mammalian cells, 53BP1 is usually expelled from the core of repair centres 4–8 h post-IR, forming a ‘cavity’ that is filled by subsequent RPA recruitment39. We thus examined, 6 h post irradiation, the morphology of 53BP1 foci and their co-localization with phospho-RPA32 foci. The appearance of cavities at the foci centres (Fig. 6f) and the recruitment of phospho-RPA32 was delayed in WDR70 knockout cells when compared with the control (Supplementary Fig. 6f). Consistent with this, depletion of 53BP1 by siRNA partially rescued the increase in NHEJ observed in WDR70 knockout cells (Supplementary Fig. 6g). Finally, in cell extracts, the RPA32

Mre11-dependent nuclease activity is required to dissociate Ku70-Ku80 from DSB ends and in the mre11Δ mutant background the inability to release Ku interferes with resection and RPA recruitment12. Concomitant Ku deletion thus restores proximal RPA recruitment in the mre11Δ mutant. Unlike the effects of crb2Δ and set9Δ, concomitant puck deletion in wdr70Δ cells did not restore ssDNA formation 3 kb from the DSB (Supplementary Fig. 5f). This indicates that Wdr70 does not participate in removing Ku from DSB ends. Taken together, these results suggest that CRL4 Wdr70-uH2B counteracts a Crb253BP1-dependent barrier to Exo1 recruitment and resection.
phosphorylation observed in response to camptothecin treatment was attenuated in WDR70 knockout cells. This attenuation was reversed by the transfection of 53BP1 siRNA (Fig. 6g). Consistent with reports that H4K20Me2 is not induced in response to DNA damage38, we did not observe changes to H4K20Me2 levels in the absence Wdr70 (Supplementary Fig. 6h). Collectively, these data are reminiscent of our observations in S. pombe: impaired DSB repair correlates with impaired Crb253BP1 repositioning and defects in RPA association with damaged DNA.

Discussion
DNA damage signalling and DNA repair occur in the context of chromatin and are modulated by multiple pathways that orchestrate spatio-temporal histone modifications to promote signalling and allow access of appropriate repair factors to DNA lesions49. A key posttranslational modification is the C-terminal phosphorylation of a H2AX (H2A in yeast), which initiates a cascade of chromatin-associated posttranslational modifications that modulate protein localization and activity in the vicinity of the DNA damage. However, not all histone modifications are dependent on prior H2AX phosphorylation. For example, H4K20 di-methylation occurs independently26,27, suggesting parallel activities converge to correctly regulate the overall DNA damage response.

DNA DSBs are the most genotoxic form of DNA damage as a single DSB can result in gross chromosomal rearrangement or cell death. Unsurprisingly, multiple DNA repair pathways have evolved to repair DSBs. HR is largely error free, while other pathways such as NHEJ and alternative micro-homology mediated end-joining pathways are intrinsically error prone. The choice of pathway used is thus at least partly dictated by the availability of an appropriate homologous template: in S phase and G2, the sister chromatid is close by due to cohesion, whereas in G1 the homologous chromosome is likely far away and thus the risk of translocation increased. Multiple mechanisms regulate the choice of repair pathway, responding to cues including the activity of cyclin-dependent kinases3,41 and the chromatin environment in which the damage arose42. Various histone modifications impact on this pathway choice, for example, in human cells H2AX-dependent RNFL-RNF168 ubiquitination of H2A promotes 53BP1 association, which inhibits DSB processing and favours NHEJ unless this inhibitory activity is countered by BRC1 (ref. 2). The parallel pathway of H2B ubiquitination by RNF20-RNF40-UbcH6 (HULCRhp6 in fission yeast) is necessary for DNA resection and efficient HR26,27. Precisely, how each of the plethora of pathways and proteins that affect DNA processing at a DSB interact is the subject of much ongoing research.

In exploring how CRL4s might promote efficient recovery from DNA damage, we uncovered evidence that CRL4Wdr70 acts as a chromatin modulator that modulates the extent of H2B monoubiquitination. In turn, this affects the extent to which DSBs are resected. We show that although uH2B occurs immediately proximal to the break site in the absence of CRL4Wdr70, the complex is necessary for distal uH2B several kilobases from the DSB site. Thus, we propose that CRL4Wdr70 does not itself modify H2B but, through presumed modification of an unknown target, acts to modulate the activity of HULCRhp6. A lack of distal uH2B, or the complete loss of uH2B, results in impaired Exo1-dependent resection. This correlates with a decrease in HR and an increase in the use of end joining pathways of repair. Intriguingly, these defects can be reversed by the concomitant deletion of Crb253BP1, which forms a barrier for resection.

Discussion DNA damage signalling and DNA repair occur in the context of chromatin and are modulated by multiple pathways that orchestrate spatio-temporal histone modifications to promote signalling and allow access of appropriate repair factors to DNA lesions49. A key posttranslational modification is the C-terminal phosphorylation of a H2AX (H2A in yeast), which initiates a cascade of chromatin-associated posttranslational modifications that modulate protein localization and activity in the vicinity of the DNA damage. However, not all histone modifications are dependent on prior H2AX phosphorylation. For example, H4K20 di-methylation occurs independently26,27, suggesting parallel activities converge to correctly regulate the overall DNA damage response.

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In exploring how CRL4s might promote efficient recovery from DNA damage, we uncovered evidence that CRL4Wdr70 acts as a chromatin modulator that modulates the extent of H2B monoubiquitination. In turn, this affects the extent to which DSBs are resected. We show that although uH2B occurs immediately proximal to the break site in the absence of CRL4Wdr70, the complex is necessary for distal uH2B several kilobases from the DSB site. Thus, we propose that CRL4Wdr70 does not itself modify H2B but, through presumed modification of an unknown target, acts to modulate the activity of HULCRhp6. A lack of distal uH2B, or the complete loss of uH2B, results in impaired Exo1-dependent resection. This correlates with a decrease in HR and an increase in the use of end joining pathways of repair. Intriguingly, these defects can be reversed by the concomitant deletion of Crb253BP1, which forms a barrier for resection.

Loss of CRL4Wdr70 function impacts on the sensitivity of S. pombe to various genotoxins including MMS and ultraviolet light, indicating that the regulation of resection is important when lesions are encountered in S phase (Fig. 1d). Unexpectedly, sensitivity to IR, which results in the majority of breaks being formed in G2 cells in fission yeast, is not increased in wdr70 mutant cells (Supplementary Fig. 2e) and no gross defects in IR-induced DSB repair are seen by pulse-field gel electrophoresis (PFGE) analysis (Fig. 1e). We speculate that this is because slow resection can be compensated for in these circumstances by an extended G2/M arrest (Supplementary Fig. 2f) that provides additional time for repair and, potentially, by the use of alternative DNA repair pathways. In human cells, depletion of Wdr70 does result in a modest sensitivity to IR (Supplementary Fig. 6e) as well as to other genotoxic agents. This suggests that the reduction of distal uH2B and the consequent attenuation of Exo1-dependent resection caused by CRL4Wdr70 loss cannot be fully compensated for in mammalian cells.

Exactly how uH2B facilitates access of the Exo1 resection machinery is unknown. In vitro, Exo1 is refractory to chromatin, unlike the BLM-Dna2 resection machinery29, and this correlates with our observation that Exo1, but not Rqh1BLM-Dna2-mediated resection, is attenuated when H2B monoubiquitination is perturbed (Fig. 4). However, we cannot explain why Crb253BP1 loss should allow access to Exo1 and restore resection in the context of defective H2B monoubiquitination. It is possible that Crb253BP1 binds tightly to chromatin, thus locking it in an inaccessible state that is reversed by uH2B. It is also currently unclear how uH2B facilitates chromatin changes: it is reported that nucleosomes in which H2B is monoubiquitinated, unlike those where H2A is monoubiquitinated, interfere with the compaction of 30 nm fibres13, potentially leading directly to a more open chromatin state. Similarly, the influence of uH2B on additional histone modifications, as is evident for H3 trans-modification during transcription44, may influence chromatin architecture. Alternatively, the ubiquitin moiety could act as a docking platform for a chromatin remodelling factor or factors that disrupt nucleosomes ahead of Exo1.

Our work adds a new player to the mechanisms that modulate long-range resection at the level of chromatin (Supplementary Fig. 6i). Using fission yeast, we show that both Ddh1, a core subunit of CRL4s, and Wdr70, one of many CRL4 adaptor proteins, interact in a DNA damage-inducible manner and have epistatic effects on resection suggesting that they can function in a common CRL4Wdr70 complex. Our analysis demonstrates that CRL4Wdr70 coordinates with HULCRhp6 (RNF20-RNF40-UbcH6) to modulate chromatin structure at DSBs and the resection of activity enzymes, although we cannot rule out additional independent roles for Ddh1 or Wdr70 in regulating DNA repair. We further demonstrate that H2B monoubiquitination is a two-step process: HULCRhp6 catalyses break-proximal uH2B on the immediate ssDNA-RPA filament formed when resection is initiated, whereas CRL4Wdr70 promotes subsequent HULCRhp6-dependent distal H2B modification. Intriguingly, H2B monoubiquitination is coordinated with changes to H4K20me2 modification. The binding of the tudor domain of Crb253BP1 to H4K20me2 is one of the mechanisms by which this inhibitor of resection is recruited to chromatin. It is postulated that H4K20 di-methylation is not synthesized de novo upon DNA damage, but is exposed upon changes to chromatin configuration33,34. It will be intriguing to clarify if uH2B affects the accessibility of H4K20me2.

In summary, our findings uncover a new and evolutionarily conserved histone regulatory module that influences DSB resection, HR and genome stability.

Methods
Uncropped blots are presented in Supplementary Fig. 7. S. pombe strains used are listed in Supplementary Table 2. Other reagents, including antibodies and primers, are listed in Supplementary Tables 3–5.
Yeast growth and molecular biology. Standard *S. pombe* growth media were used45. Gene disruption and TAP-, HA- or MYC-tagged strains were created using PCR-based HR46. Cells were grown in YEA or EMM-media supplemented with essential amino acids unless stated otherwise. Cell cycle synchronization was achieved by arresting cdc25-22 cells at 37°C for 6 h. G2 phase-arrested cells were subsequently released at 25°C by quick cooling in ice water45. Septation was determined by fixing cells in methanol and staining with 4',6-diamidino-2-phenylindole (DAPI) and calcofluor45. The *wdr70* gene was amplified from an *S. pombe* cDNA library (primers 27/28) and cloned into the SalI/BamHI sites of RepE1-EGFP using an infusion PCR kit (Clontech, ST0345). PFU polymerase (STRATAGENE, 600252-52) was used for site-directed mutagenesis (W252A D326K; primers 25/26) in RepE1-EGFP-Wdr70.

Survival assays. To measure the sensitivity to chronic exposures to genotoxic agents, prelogarithmic cultures were harvested, resuspended in fresh medium at 1 x 10^5 per ml and diluted tenfold. 10 μl aliquots serially of these dilutions were spotted onto YEA agar plates containing indicated amounts of CPT or MMS. Representative pictures were chosen from two independent analyses. For exposure to IR, cells were irradiated using a Cs source or custom-made X-ray machine (Wandel & Goltermann). Plates were incubated and plated and then treated onto YEA plates. In the case of ultraviolet treatment, cells were serially diluted and spotted onto YEA plates and irradiated using a Stratagen Stratalink ultraviolet source. All plates were incubated for 4–5 days at 30°C before being photographed. Colony formation assays were carried out using the same genotoxic agents, except that 500–1,000 cells were counted on serial dilutions of 10% SDS for colony numbers after incubation at 30°C for 10 days. Three independent analyses were performed and each time included three plates for each concentration of drugs or radiation dosage.

Whole-cell lysates (WCE) from *S. pombe* and western blotting. For WCE, overnight logarithmically growing yeast cells in rich medium were harvested either before or after the indicated treatment with genotoxic agents or after HO induction. Twenty OD_{600} of wild-type cultures were pelleted and dispersed in 200 μl 0.3 M NaOH for 10 min at room temperature. Two volumes of 20% trichloroacetic acid solution was added to lysed cells to precipitate protein followed by centrifugation at 20,000g for 10 min. Pellets were resuspended in SDS sample loading buffer (pH8.0) and boiled for 5 min. Samples were centrifuged for 1 min at 20,000g and supernatant was retained as WCE46. Boiled WCEs, histone extract or immunoprecipitated samples were resolved by 8–15% SDS–PAGE and transferred by centrifugation at 20,000g for 10 min. Supernatant was transferred to new tube and nucleic acids precipitated using 0.10 mol per liter isopropanol. The pellet was washed with 70% ethanol. DNA was air dried and resuspended in double distilled water plus 5 μl of 10 mg/ml RNase A. Aliquots were incubated at 37°C for 1 h and 0.2 μg of 5′ and 20 μg of 20 mg/ml proteinase K were added and the solution was incubated overnight at 30°C. The solution was then extracted twice with equal volume of phenol/chloroform (1:1) and DNA precipitated using isopropanol. The pellet was washed with 70% ethanol, air dried and was resuspended in 100 μl ddH₂O.

Analysis of ssDNA processing by Apol digestion. Strains used in resection analysis harbour a single HO cleavage site at the arg5 locus and a gene coding for homologous recombination under the control of nmt41 promoter. For induction of HO endonuclease, pre-cultures were grown over-night in the presence of thiamine, washed with 70% ethanol and resuspended in 2× SPS medium supplemented with 10% yeast extract and adenine. Cells were then grown at 30°C to mid-log phase and divided into two fractions. One was used for induction by removing thiamine while the second was used for induction from 37°C to 28°C. Induction was performed by shifting cultures back to 30°C at 14–16 h following thiamine removal. Resection was measured at 35 bp and 5 kbp from the break based on the location of the Apol restriction sites. After induction of HO endonuclease by removing thiamine, at the indicated times, genomic DNA was extracted from 50 OD_{600} of culture following digestion with Apol (Fermentas, #ER1381). The overall rate of resection was assessed by semi-quantitative or real-time quantitative PCR (qPCR)49 (35 bp; primer pair 1); 3 kb; primer pair 13; 5.4 kb; primer pair 19; 9 kb; primer pair 21. Induction of resection was assessed by measuring the percentage of uncleaved DNA using PCR with primer pair 23/24.

Chromatin immunoprecipitation. Following 15 min cross-linking for formaldehyde at a final concentration of 1%, cells were harvested, washed once in PBS and broken in a Ribolysis with glass beads in genomic DNA extraction buffer. Extracts were sonicated to yield DNA fragment at a size between 500 and 1,000 bp. Rabbit IgG-coated magnetic beads were used to retrieve TAP-tagged proteins. Immune-complexes retrieved using anti-uH2B and anti-H4K20-dimethyl antibodies were enriched by Protein G magnetic Dynabeads. Cross-linking was reversed by incubation at 65°C for 2 h, DNA was purified using a Gel Extraction Kit (Omega, D2500-02). 35 ng of recovered DNA was used for each quantitative real-time PCR reaction14–16. For each data point, all reactions were performed in triplicate. The primers used to measure protein enrichment: 0.2 kbp, primer pair 17/18; 3 kb, primer pair 15/16; 5.4 kbp, primer pair 19/20; 9 kb, primer pair 21/22. Reactions were run on CFX-96 real-time system (Bio-Rad) using Promega GoTaq qPCR Master Mix.

Transcription analysis by RNA sequencing. Cells were arrested in G2 using the cdc25.22 allele. wdr70^-1 and wdr70A strains (two biological replicates per condition) were subjected to total RNA extraction and mRNA was purified using poly-T two-strand magnetic beads (NEN/GE Healthcare). Sequencing libraries were generated from purified PolyA + RNA using a NENNext Ultra RNA Library Prep Kit for Illumina sequencing an Illumina HiSeq2500 platform following the manufacturer’s protocol. 125 bp/150 bp paired-end reads were generated. HTSeq v0.6.1 software was used to count the reads and numbers mapped to each gene. Differential expression analysis between HO^+ and HO^- strains was performed using the DESeq R package (1.18.0). Corrected P-value of 0.005 and log2 (Fold change) of 1 were set as the threshold for significantly differential expression.

Human cell culture and analysis. Human cell lines were maintained in culture media supplemented with 10% fetal bovine serum. HEK293T obtained from...
American Type Culture Collection or their derived cell lines were cultured in DMEM. All cell lines tested negative for mycoplasma. Lentivirus expressing 3BP1DNA GV282 and 53BP1 (target sequence: 5’-TGTCGTTCAAGCGGACCTTIT-3’) was packaged by co-transfecting 293T cells with pLVX-ires-ZsGreen, pMD.2G and pSPAX2 plasmids using X-tremeGene HE (Roche, 06366244001). Secreted virus was collected 72 h after transfection. Infection of supernatant was determined by positive green fluorescent protein clones in 96-well plates at tenfold serial dilution and cells were infected at a titre of 2 × 10^4 (multiplicity of infection) per well. Cells with stably integrated lentivirus were propagated in DMEM supplemented with 10% fetal bovine serum up to 1 month for experimental use.

Single duplex siRNA used in this study were purchase from Rhobio. Individual siRNA duplexes used were: DDB1 (siRN593141904, target sequence: 5’-CCU GGUGAUUGCCCACCAAGUG-3’), DDB2 (siRN717103883, 5’-CUCCUGAGGACAGGCUA-3’), WDR70 (001: siRN711130927, target sequence: 5’-CUCCCGAGAAGGACGGCAACU-3’; 002: siRN711103988, target sequence: 5’-GAGCAAAAUGGUGGAGCAGAGA-3’). Transfections were performed using Lipofectamine 2000 or X-tremeGene siRNA Transfection Reagent (Roche, 04476093001) following the manufacturer’s instruction. Normally, two parallel technical repeat samples. Statistical analysis was performed in Microsoft Excel using the Student’s t-test if two groups are compared. Outliers were eliminated by Q-test if Q > Q_{0.05}.

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**Author contributions**

C.L. and A.M.C. designed genetic and cellular assays. M.Z., K.M. and K.N. performed the yeast experiments. H.W. and L.G. participate in molecular cloning and statistical analysis. Z.T., X.W. and L.R. analysed human CRL4*WDR70*. C.L. and A.M.C. summarized data from all contributors and wrote the paper.

**Additional information**

**Accession codes:** The RNA-seq data have been deposited in GEO repository under the accession codes GSE79830.

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