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Cmr1/WDR76 defines a nuclear genotoxic stress body linking genome integrity and protein quality control

Irene Gallina1, Camilla Colding1, Peter Henriksen2, Petra Beli2,†, Kyosuke Nakamura3, Judith Offman4,†, David P. Mathiasen1, Sonia Silva1, Eva Hoffmann4, Anja Groth3, Chunaram Choudhary2 & Michael Lisby1

DNA replication stress is a source of genomic instability. Here we identify changed mutation rate 1 (Cmr1) as a factor involved in the response to DNA replication stress in Saccharomyces cerevisiae and show that Cmr1—together with Mrcl/Claspin, Pph3, the chaperonin containing TCP1 (CCT) and 25 other proteins—define a novel intranuclear quality control compartment (INQ) that sequesters misfolded, ubiquitylated and sumoylated proteins in response to genotoxic stress. The diversity of proteins that localize to INQ indicates that other biological processes such as cell cycle progression, chromatin and mitotic spindle organization may also be regulated through INQ. Similar to Cmr1, its human orthologue WDR76 responds to proteasome inhibition and DNA damage by relocalizing to nuclear foci and physically associating with CCT, suggesting an evolutionarily conserved biological function. We propose that Cmr1/WDR76 plays a role in the recovery from genotoxic stress through regulation of the turnover of sumoylated and phosphorylated proteins.

1 Department of Biology, University of Copenhagen, Room 4.1.07, Copenhagen N DK-2200, Denmark. 2 The Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen N DK-2200, Denmark. 3 Biotech Research and Innovation Centre (BRIC) and Centre for Epigenetics, University of Copenhagen, Copenhagen N DK-2200, Denmark. 4 MRC, Centre for Genome Damage and Stability, School of Life Sciences, University of Sussex, Brighton BN1 9RH, UK. † Present addresses: Institute of Molecular Biology (IMB), 55128 Mainz, Germany (P.B.); Centre for Cancer Prevention, Wolfson Institute of Preventive Medicine, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, UK (J.O.). Correspondence and requests for materials should be addressed to M.L. (email: mlisby@bio.ku.dk). DOI: 10.1038/ncomms7533
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ithful completion of DNA replication is essential for cell survival and for inheritance of the genetic information. Replication fork stalling at DNA lesions leads to activation of the replication checkpoint, which in S. cerevisiae relies on the recruitment of the checkpoint kinase Mec1/ATR to RPA (replication protein A)-coated single-stranded DNA, arising from the uncoupling of the polymerase and the mini-chromosome maintenance (MCM) helicase1. Mec1-dependent phosphorylation of the checkpoint mediator Mrcl/Claspin leads to the recruitment and activation of the effector kinase Rad53 (refs 2,3). The replication checkpoint induces posttranslational modification of the clamp loader PCNA (proliferating cell nuclear antigen), promoting the repair or bypass of the lesion4. Failure to activate the replication checkpoint leads to severe chromosomal instability, a major trigger for cancer in humans5.

Resolution of DNA replication after checkpoint activation relies both on the repair or bypass of the lesion and on the inactivation of checkpoint signalling. The latter requires dephosphorylation of Rad53 by the PP4 phosphatase Pph3-Psy2-Psy4 (ref. 6) and proteasome-dependent degradation of fork-associated factors such as Mrcl (ref. 7). Specifically, Mrcl has recently been identified as a target of the ubiquitin ligase complex SCF–Dia2 (ref. 7). Dia2 directly binds Mrcl and promotes its ubiquitylation and proteasomal degradation in response to replication stress. This recovery pathway appears to act in parallel with dephosphorylation of Rad53, as DIA2 and PPH3 show negative genetic interaction in the presence of replication stress8.

Cmr1 (changed mutation rate 1) is a nuclear WD40 protein of unknown function9,10, which has recently appeared in several large-scale studies. First, Cmr1 was described as a histone-related protein11, with DNA-binding capacity in vitro and with the ability to accumulate on chromatin in response to ultraviolet irradiation12. Furthermore, in a genome-wide screen Cmr1 was found to specifically respond to methyl methanesulfonate (MMS)-induced damage, relocating to nuclear foci of undetermined nature13. Finally, in silico clustering analyses suggest that CMR1 is co-expressed with genes involved in processes related to DNA metabolism14. Taken together, these data suggest a role for Cmr1 in genome maintenance. Here we identify Cmr1 in two independent screens and provide the first extensive functional characterization of Cmr1 and the nuclear structure that it forms in response to replication stress and proteasome inhibition. Together with the replication checkpoint proteins Mrcl, Pph3 and 25 other proteins, Cmr1 defines a novel intranuclear quality control compartment (INQ) for the sequestering of phosphorylated, sumoylated and ubiquitylated proteins. Our findings document a novel connection between the cellular response to DNA replication stress and turnover of replication stress factors.

Results

Identification of Cmr1 as a genome maintenance factor. In an effort to identify new factors involved in the maintenance of genome stability, a series of stable isotope labelling by amino acids in cell culture (SILAC)-based mass spectrometry (MS) experiments were performed under conditions wherein the replication protein Rfa1 and the recombination protein Rad52 were induced to relocalize to DNA repair foci by DNA damage before protein extraction and pull down using a yellow fluorescent protein (YFP) tag (Fig. 1a). This approach identified a collection of proteins, including the WD40-domain protein Cmr1 (Fig. 1b). Further, the physical association between Cmr1 and the RPA complex, which has been reported in several independent large-scale studies1,15,16, was confirmed by reverse pull down using Cmr1-YFP as the bait (Fig. 1c and Supplementary Data 1).

In an independent systematic genome-wide screen for mutants that change mutation rates, we found that cmr1Δ suppressed the otherwise elevated mutation rates resulting from expression of the human mismatch repair (MMR) gene MLH1 (hMLH1) in S. cerevisiae (Supplementary Fig. 1)17. Further analyses to assess the involvement of Cmr1 in MMR showed that deletion of CMR1 increases frameshift mutation rates in an MRC1- and MLH1-dependent manner, but additively increases the overall CAN1 forward mutation rate in conjunction with msh2Δ and mlh1Δ (Table 1a,b). These findings indicate a defect in replication rather than in MMR per se.

Cell cycle-independent formation of perinuclear Cmr1 foci. The observation that endogenously tagged Cmr1 localized to diffusely nuclear to a distinct focus on hMLH1 expression in yeast or after treatment with MMS, ultraviolet irradiation or hydroxyurea (HU;Fig. 1d–f), supported our hypothesis of Cmr1 being recruited to a replication or DNA repair factory. Surprisingly, but consistent with a recent report13, the Cmr1 focus did not co-localize with any known nuclear structures such as telomeres (Cdc13), nuclear pore complex (Nup49), MMR (Pms1), spindle pole body (Spcl10), recombination (Rad52), replication (Pol30 and Rfa1), or the nucleolus (Nop1, data not shown). We therefore concluded that Cmr1 defines a novel nuclear compartment that forms in response to genotoxic stress.

Given the vicinity of Cmr1 foci to the nuclear periphery, we further examined Cmr1 foci relative to the nuclear membrane in asynchronously growing and G1-arrested cells. Cmr1 foci formed with similar efficiency in G1 and S/G2 cells, consistently localized internally to the nuclear envelope (Nup49-CFP), and disassembled within 90 min after ultraviolet irradiation or on removal of MMS (Fig. 2a–c and Supplementary Fig. 2a,b). Accumulation into perinuclear foci was also observed for the endogenous Cmr1 (Supplementary Fig. 2a,b,d), indicating that its localization is not an artefact of the YFP tagging. Structurally, Cmr1 is predicted to consist of a carboxy-terminal WD40 domain and of an amino-terminal unstructured region. Using plasmids expressing YFP fusions of the N-terminal domain (NTD) or the WD40 domain, we found that the WD40 domain was necessary and sufficient for the re-localization of Cmr1 into foci (Fig. 2d,e).

Cmr1 marks an intranuclear quality control compartment. To gain insight into the biological processes represented by Cmr1 foci, we screened a collection of 4800 green fluorescent protein (GFP)-tagged proteins for co-localization with Cmr1 (ref. 10). We took advantage of the observation that Cmr1 perinuclear foci were also induced by proteasome inhibition (MG132; Supplementary Fig. 2e), to avoid induction of DNA repair foci13. Strains exhibiting MG132-induced perinuclear foci were individually re-tested for co-localization with Cmr1-YemRFp, yielding a list of 27 proteins that form Cmr1-co-localizing foci in response to MG132 (Fig. 3a). Eighty-one per cent (22/27) of these proteins also co-localized with Cmr1 after MMS treatment. Notably, proteins implicated in chromosome organization, mitotic cell cycle, spindle organization and dephosphorylation were overrepresented among these proteins (Supplementary Fig. 3a–c). The hits included regulators of the S-phase checkpoint response (Mrcl and Pph3) and components of the anaphase-promoting complex (Cdc20, Cdc27 and Apc4), chaperones (Hsp104 and Apj1) and histone deacetylases (Hos2 and Rpd3)13.

To further characterize the properties of Cmr1 foci, we screened the collection of 5,200 non-essential gene deletion mutants18 to determine the genetic requirements for the
formation of Cmr1 foci in the presence of replication stress, and to identify gene deletions that would lead to spontaneous accumulation of Cmr1 foci. Consistent with their induction by proteasome inhibition, the top-scoring hits for increased accumulation of Cmr1 foci, indicating that the proteasome has the potential to target proteins for degradation at the perinuclear structure defined by Cmr1 (Supplementary Fig. 3d).

The only non-essential genes required for Cmr1 focus formation were HSP42 and BTN2 (Fig. 3c). Hsp42 is a small heat shock protein with chaperone activity, which has recently been found to be essential for organization and sorting of protein aggregates into deposition sites in yeast. Similarly, Btn2 has been identified in a recent study as a crucial regulator of the cellular protein quality control. Interestingly, both Hsp42 and Btn2 have been implicated in the partitioning of misfolded proteins for degradation at the perinuclear structure defined by Cmr1 (Fig. 3d).

Figure 1 | Cmr1 associates with RPA-bound chromatin. (a) Representation of the workflow used for the SILAC-based identification of protein complexes with DNA repair factors. Yeast strains expressing YFP-tagged (IG54-11D and IG46-1B) or untagged (IG45-8A) proteins were cultured in SILAC media and harvested in log phase after treatment with DNA-damaging agents. Protein complexes from SILAC lysates were affinity purified separately with GFP-Trap. Proteins were trypsin proteolysed and peptides were identified by liquid chromatography (LC)–MS/MS. (b) Identification of Rfa1-YFP and Rad52-YFP interacting proteins. The plots show log(10) SILAC ratios from GFP-tagged bait versus control from forward and reverse (SILAC label swap) experiments. Dots indicate identified proteins. Cmr1 is highlighted in red and some of the known interactors for Rfa1 and Rad52 are indicated in black in the respective plots. (c) Identification of Cmr1 interacting proteins. Cmr1-YFP (IG71-2B) was used as bait for the pull down; Cmr1 is highlighted in red; CCT-chaperonin complex subunits and Rfa1 are indicated in black. (d) Cmr1 relocalization into foci. Representative images of untreated and MMS-treated cells are shown. Arrowheads indicate selected foci. Scale bar, 2 μm. (e) Quantification of Cmr1 foci. Cmr1-YFP localization was examined by fluorescence microscopy in IG66. Cells were grown to exponential phase and imaged after treatment with zeocin (200 μg ml⁻¹), MMS (0.05%), CPT (5 μg ml⁻¹), 4-NQO (0.2 μg ml⁻¹), HU (200 mM), EMS (0.5%) for 2 h, or 1 h after ultraviolet irradiation (25 J m⁻²). Error bars represent 95% confidence intervals. Two to 3 replicates of 100–200 cells were analysed for each condition. (f) hMLH1 expression causes accumulation of Cmr1 foci. Cells expressing Cmr1-YFP (IG66) were transformed with pEH333 for ectopic expression of hMLH1 or with an empty vector (pEH334). Error bars represent 95% confidence intervals. Two to 3 replicates of 100–200 cells were analysed for each strain.
To assess whether Cmr1 foci coincide with JUNQ, the localization of the unassembled von Hippel–Lindau (VHL) tumour suppressor VHL-GFP and Cmr1-yEmRFP was monitored under conditions leading to VHL misfolding and accumulation into deposition sites. Indeed, Cmr1 co-localized with VHL at the nuclear periphery in >50% of the cells, but was never observed at the perivacuolar IPOD. As Cmr1 foci resemble JUNQ but are strictly nuclear, we name this structure intranuclear quality control (INQ). In agreement with our observation that INQ is a nuclear structure, we observed that >95% of the nuclear mCherry-VHL foci co-localized with Cmr1, while none of the juxtanuclear or peripheral cytoplasmic VHL foci co-localized with Cmr1 (Fig. 3d,e), indicating that INQ is a nuclear variant of JUNQ that can be distinguished by the presence of Cmr1. Notably, MGI321-induced nuclear VHL foci only required BTN2, while MMS-induced VHL foci also required HSP42, indicating that the sorting of Cmr1 and VHL to INQ could occur by different mechanisms (Supplementary Fig. 4b,c).

The physical interaction of Cmr1 with all eight subunits of the chaperonin containing TCP1 (CCT) (Fig. 1c) prompted us to examine the recruitment of CCT to INQ. On expression of Cct6-YFP, ~60% of the cells exhibited one to three cytoplasmic and nuclear Cct6 foci, typically one very bright focus in the cytoplasm and one weaker focus at the nuclear periphery (Fig. 3f). Importantly, ~12% of the Cct6-YFP perinuclear foci co-localized with Cmr1, while no co-localization was observed with the brighter cytoplasmic focus. Notably, Cmr1 itself did not behave similar to a misfolded protein, as its relocation to INQ was not perturbed by the actin or microtubule depolymerizing drugs latrunculin B and nocodazole, respectively, a requirement previously demonstrated for relocation of misfolded proteins to JUNQ (Supplementary Fig. 4d,e and data not shown) and/or refolding of proteins primarily involved in DNA metabolism and cell cycle control.

Cmr1 interacts with chromatin and replication factors. As Cmr1 relocation was not coupled to its degradation, we reasoned that Cmr1 might facilitate another aspect of INQ function. To identify possible targets of Cmr1 function, we performed a systematic genome-wide screen for in situ physical interactions using bimolecular fluorescence complementation (Fig. 4a).

A query strain expressing Cmr1 fused to the C-terminal fragment of Venus (Cmr1-VC) was crossed to a library of 79 proteins previously demonstrated for relocalization of misfolded proteins to INQ (Supplementary Fig. 4d,e and data not shown) and/or refolding of proteins primarily involved in DNA metabolism and cell cycle control.

### Table 1 | Effect of Cmr1 on mutation rates and chromosome loss.

<table>
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<tr>
<th>Genotype</th>
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<th>Mutation rate (fold change)</th>
<th>Chromosome loss (fold change)</th>
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<td>Lys ( \times 10^{-2} )</td>
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<td>4.7 (1.2)</td>
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<td>mhl1Δ</td>
<td>IG106-5A</td>
<td>63 (17)†</td>
<td>77420 (17204)†</td>
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<td>23790 (5278)†</td>
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<td>IG137-66D</td>
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<table>
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<td>99 (2.5)†</td>
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<td></td>
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<td>6.6 (2)†</td>
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<td>SMG2-9-3C × SMG2-9-11B</td>
<td>7.5 (2.3)†</td>
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<td>IG156-9B × IG156-9C</td>
<td>1.9 (4.8)†</td>
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BM, bimaters assay; MMS, methyl methanesulfonate.

**a.** Spontaneous mutation rates. Forward (CanR) and frameshift (Lys) mutation rates were determined for IG106-4D (wt), IG106-1C (cmr1Δ), IG106-5A (mhl1Δ), IG106-1D (cmr1Δ mhl1Δ), IG137-66D (msh2Δ), IG137-28C (msh2Δ cmr1Δ), IG172-7C (mrc1Δ) and IG172-4B (mrc1Δ cmr1Δ). **b.** Induced mutation frequencies. Fold change compared with wild type is indicated in parentheses. Replication stress-induced mutation frequencies were determined after growth in 0.03% MMS for 30 min. **c.** Rates of chromosome loss (BiM assay). Diploid strains homozygous for the indicated gene deletions were grown on rich medium and subsequently tested for the ability to mate with a MATα tester strain (G133). Frequency of mating, derived from loss of the endogenous MATα locus, is used as a measure of chromosomal instability. The two sets of BiM assays were performed with different batches of media; s.d. of the rate is reported.

*Relative to wild type.
†Significant \(P<0.05\) compared with wild type.
‡Significant \(P<0.05\) difference between single and double mutant.
to be unique interactors of Cmr1 compared with other bait proteins tested (Supplementary Table 1 and unpublished data). These showed a significant overrepresentation of Gene Onthology biological process terms related to DNA replication, transcription and regulation of gene expression (Fig. 4b), consistent with the notion that Cmr1 is a component of chromatin. However, none of the other INQ factors were found to interact with Cmr1.

The VN–VC interaction signals were mainly nuclear and a subnuclear localization pattern into foci or speckles was often observed (Supplementary Fig. 5). Interestingly, four replication fork proteins, Mcm3, Pri1, Rfc2 and Rfc3, were identified in this screen (Fig. 4c,d and Supplementary Fig. 5). Notably, the Mcm3–Cmr1 interaction significantly increased by about twofold in response to MMS treatment (Fig. 4d). Taken together, this analysis confirms that Cmr1 interactions are enriched for chromatin-associated factors.

**Btn2 promotes Mrc1 turnover and relocalization to INQ.**

Given the interaction of Cmr1 with replication fork components, and based on our observation that the replication checkpoint mediator Mrc1 localizes at INQ (Fig. 3a), we further addressed the functional relationship between Cmr1 and Mrc1. Mrc1-CFP formed foci in response to DNA-damaging agents with a nearly identical profile to that of Cmr1-YFP (Supplementary Fig. 6a), and the two proteins mostly co-localized after MMS or MG132 treatment (Fig. 5a,b). Nevertheless, the ability of Cmr1 or Mrc1 to form foci occurred independently of each other (Supplementary Fig. 6b,c).

Following replication checkpoint activation, Mrc1 is phosphorylated at (S/T)Q sites, which stimulates its degradation via Dia2 and possibly other factors, to promote recovery from replication stress. To establish whether there is a correlation between the degradation of Mrc1 and its relocalization to INQ, we took advantage of the replication-proficient but checkpoint-defective separation-of-function mutant mrc1AQ. The mrc1AQ mutant showed constitutively higher levels of Mrc1 protein due to a partial defect in Dia2-mediated degradation (Fig. 5c,d). After treatment with MMS, the Mrc1AQ protein was partially defective in relocation to INQ (Fig. 5e) and Mrc1 foci were almost

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**Figure 2 | Cmr1 localizes into perinuclear foci.** (a) Cmr1 foci localize at the nuclear periphery. Cells expressing Cmr1-YFP (IG66) were transformed with pNEB21 for expression of Nup49-CFP and pML84 for expression of NLS-yEmRFP, and treated with 0.05% MMS. Cmr1 localization relative to the nucleoplasm and the nuclear membrane is shown in both unbudded (G1) and budded (S/G2) cells. Nuclear zooms are at 150%. (b) Cmr1 foci in α-factor-arrested cells. Cells (IG66) were cultured with α-factor for 1 h, followed by treatment with 0.05% MMS for 2 h. (c) Quantification of Cmr1 foci. The percentage of cells (IG66) with Cmr1-YFP foci was quantified in asynchronous and G1-arrested populations, before and after treatment with 0.05% MMS for 1 h, and 30, 60 and 90 min after the drug was removed. Two to 3 replicates of 100–200 cells were analysed for each condition. Error bars represent 95% confidence intervals. (d) Schematic representation of Cmr1 and truncation constructs. (e) The WD40 domain of Cmr1 is necessary and sufficient for relocalization into foci. Cmr1-YFP constructs containing the full-length protein (pIG13), the N-terminal (pIG14) or WD40-containing portion of the protein (pIG15) were expressed from a 2-μm plasmid in cells (ML657) harbouring NLS-yEmRFP, and relocation of Cmr1 into nuclear foci was monitored after treatment with 0.05% MMS. Scale bars, 2 μm.
Figure 3 | Characterization of INQ. (a) Genome-wide analysis of Cmr1 co-localizing proteins. Haploid cells expressing GFP-tagged query proteins and Rad52-RFP as a nuclear marker (IG72-5C) were imaged by high-content fluorescence microscopy, untreated or treated for 2 h with 75 μg ml⁻¹ MMS or CHX. Proteins re-localizing into perinuclear foci were further tested for co-localization with Cmr1. Confirmed co-localizing proteins are listed. Proteins that also co-localize with Cmr1 after MMS treatment are highlighted in bold. (b) Cmr1 foci are induced by genetic impairment of proteasome function. Gene deletion strains expressing Cmr1-YFP and NLS-yEmRFP were imaged by high-content fluorescence microscopy. Strains exhibiting more than threefold increase in the percentage of spontaneous Cmr1 foci compared with wild type were manually retested. Only the mutants giving a result significantly different from the wild-type reference strain for the manual re-testing. Red and blue dashed lines represent the threefold thresholds for the automated and manual analyses, respectively. IG66 served as the wild-type reference strain for the manual re-testing. (c) Cmr1 foci are dependent on Hsp42 and Btn2. Cells deleted for hsp42Δ and btn2Δ were added. Cmr1-TAP and tubulin were analysed by immunoblotting, using β-actin as a loading control. (d) Cmr1 foci are dependent on Hsp42 and Btn2. Cells deleted for hsp42Δ and btn2Δ were added. Cmr1-TAP and tubulin were analysed by immunoblotting, using β-actin as a loading control. (e) Co-localization of Cmr1, VHL, and Nup49. (f) Cmr1 foci are induced by genetic impairment of proteasome function. Gene deletion strains expressing Cmr1-YFP and NLS-yEmRFP were imaged by high-content fluorescence microscopy. Strains exhibiting more than threefold increase in the percentage of spontaneous Cmr1 foci compared with wild type were manually retested. Only the mutants giving a result significantly different from the wild-type reference strain for the manual re-testing. Red and blue dashed lines represent the threefold thresholds for the automated and manual analyses, respectively. IG66 served as the wild-type reference strain for the manual re-testing. (g) Cmr1 foci are induced by genetic impairment of proteasome function. Gene deletion strains expressing Cmr1-YFP and NLS-yEmRFP were imaged by high-content fluorescence microscopy. Strains exhibiting more than threefold increase in the percentage of spontaneous Cmr1 foci compared with wild type were manually retested. Only the mutants giving a result significantly different from the wild-type reference strain for the manual re-testing. Red and blue dashed lines represent the threefold thresholds for the automated and manual analyses, respectively. IG66 served as the wild-type reference strain for the manual re-testing.
completely absent in a dia2Δ mutant (Supplementary Fig. 6d). Similarly, abolishing INQ through deletion of BTN2 or HSP42 prevented Mrcl focus formation (Fig. 5g) and btn2Δ led to a significant decrease in the turnover of Mrcl with or without replication or proteasomal stress (Fig. 5h,i), as measured using a fluorescence timer construct, consisting of a fast-maturing GFP and a slow-maturing mCherry. Together, these data support a functional relationship between Mrcl turnover during replication stress and relocalization of the protein to INQ.

cmr1Δ suppresses mutations in MRC1, CTF18 and PPH3.

In parallel to checkpoint mechanisms, several other pathways contribute to replication stress tolerance including homologous recombination, translesion synthesis, template switching and replication fork stabilization and restart (reviewed in ref. 27), and mutants in these pathways display different degrees of sensitivity to replication stress. The partial redundancy among these pathways may explain the lack of pronounced MMS, HU and ultraviolet irradiation sensitivity of the cmr1Δ mutant (see below and ref. 12). Hence, to uncover the epistatic relationship between CMR1 and known replication stress tolerance pathways, we performed a genome-wide screen for CMR1 genetic interactions in the presence of replication stress (HU) using the synthetic genetic array (SGA) approach29. Differential growth on HU-containing plates between the single and double mutants was assessed using ScreenMill29 and revealed negative genetic interactions of CMR1 with genes in the homologous recombination pathway (RAD50, RAD55 and MMS4), and suppression of defects associated with deletion of MRC1 and TOF1 of the replication-pausings checkpoint complex (Supplementary Tables 2 and 3). Consistently, cmr1Δ additionally increased spontaneous chromosome loss in a rad52Δ mutant and suppressed the high chromosome loss rates of an mrc1Δ mutant (Table 1c). Additional manual testing further showed that deletion of CMR1 was able to suppress the MMS and HU sensitivity of pph3Δ (PP4 phosphatase subunit) and ctf18Δ (alternative clamp loader) mutants (Fig. 5j). Importantly, suppression of the MMS sensitivity of the ctf18Δ mutant by cmr1Δ probably reflected a suppression of the DNA replication checkpoint defect but not the cohesion defects associated with this mutant29–32, as the severe defect in sister chromatid cohesion in the ctf18Δ mutant was not alleviated by cmr1Δ (Supplementary Fig. 7b). Notably, hsp42Δ was epistatic with cmr1Δ for suppressing the DNA damage sensitivity of ctf18Δ, suggesting that relocalization of Mrcl to INQ is required for Mrcl function in replication stress tolerance (Supplementary Fig. 7a). Consistent with the increased rate of chromosome loss in the rad52Δ cmr1Δ mutant compared with the single mutants, cmr1Δ displayed a negative genetic interaction with both rad52Δ and mre11Δ for survival on MMS (Fig. 5k), indicating a requirement for Mrcl in the absence of functional homologous recombination. Given that no genetic interactions were observed with genes involved in template switching (rad5Δ), MMR (msh2Δ, msh6Δ and pms1Δ), post-replicative repair (rad18Δ, mms2Δ and mms22Δ) or translesion synthesis (rev3Δ; Supplementary Fig. 7c–e and Supplementary Table 2), these data suggest that Mrcl either acts as a negative regulator of a factor required for HU resistance in the absence of Mrcl-Ctf18-Pph3 or promotes a pathway that is toxic in mutants of Mrcl-Ctf18-Pph3.

Cmr1 promotes DNA-damage checkpoint adaptation. The negative genetic interaction of cmr1Δ with mutations in
homologous recombination genes and the suppression of mutations in DNA damage and replication checkpoint genes could be due to Cmr1 promoting replication restart or regulating checkpoint recovery. To directly assess the involvement of Cmr1 in replication fork restart, we released cells from a G1 arrest into S phase in the presence of MMS for 45 min and subsequently monitored completion of DNA synthesis by flow cytometry after MMS removal. As expected, wild-type cells accumulated in S phase in the presence of MMS and slowly recovered from the blockage when the drug was removed (Supplementary Fig. 7f,g). *cmr1Δ* cells were proficient in replication checkpoint activation and restart of DNA synthesis compared with wild type. In contrast, a *dia2Δ pph3Δ* mutant was extremely sensitive to MMS and severely defective in replication restart after removal of MMS (Fig. 6a and Supplementary Fig. 7f,g). Notably, *cmr1Δ* partially suppressed the MMS sensitivity of the *dia2Δ pph3Δ* mutant without suppressing the replication restart defect, indicating that Cmr1 is not acting directly on replication restart. Moreover, also *btn2Δ* and *hsp42Δ* partially suppressed the MMS sensitivity of the *dia2Δ pph3Δ* mutant, suggesting that the suppression conferred by *cmr1Δ* is functionally related to its accumulation at INQ. As Pph3 and Dia2 are regulators of the DNA damage and replication checkpoints, respectively, we sought to test whether the suppression of *dia2Δ pph3Δ* MMS sensitivity by *cmr1Δ* is due to a role of Cmr1 in checkpoint adaptation, a mechanism by which cells deactivate the checkpoint after prolonged exposure to DNA damage and replication stress.

**a** Mrc1-YFP  
**b** Mrc1-CFP  
**c** YFP  
**d** Intensity of nuclear signal (AU)  
**e** Cells with foci (%)  
**f** Bright field  
**g** Cells with Mrc1 foci (%)  
**h** Mrc1 mCherry sfGFP  
**i** GFP/mCherry intensity (fold difference)  
**j** Wild type  
**k** Wild type
damage. To assess the impact of Cmr1 and INQ on the DNA damage checkpoint, we performed a checkpoint adaptation assay using the cdc13-1 allele, which causes uncapping of telomeres and DNA-damage checkpoint activation at the restrictive temperature. We included in the assay a mutant of the INQ component Rpd3, which has previously been reported to be adaptation defective. After growth at the restrictive temperature for 24 h, the number of cell bodies was counted. This assay indicated that cmr1Δ and, to a lesser extent, rpd3Δ, bkn2Δ and hsp42Δ are adaptation defective (Fig. 6b). In contrast, an exo1Δ mutant, which reduces resection of uncapped telomeres, rescued the cdc13-1 temperature sensitivity as described previously. Adaptation has previously been linked to Rad53 activity. We therefore examined the electrophoretic mobility shift of phosphorylated Rad53 in the same adaptation assay. cdc13-1 cells grown at the restrictive temperature for 6 h showed elevated levels of Rad53 phosphorylation, which is completely abolished in the wild type at 24 h, when adaptation has occurred. Strikingly, Rad53 remained partially phosphorylated at the 24 h time point in the cmr1Δ, bkn2Δ and hsp42Δ mutants, which is consistent with the adaptation defect of these mutants (Fig. 6c). Taken together, these data indicate that INQ promotes DNA-damage checkpoint adaptation through attenuation of Rad53 phosphorylation.

Sumoylated proteins localize at INQ. Sumoylation has previously been implicated in checkpoint regulation and a strong negative genetic interaction of cmr1Δ was observed with mutation of the SLXS-SLXS small ubiquitin-like modifier (SUMO)-targeted ubiquitin ligase (STUbL; Fig. 6d and Supplementary Table 2). This raised the possibility that sumoylation could be a signal for proteins to be channeled to the proteasome via INQ and Cmr1. This prompted us to examine the localization of sumoylated proteins in cmr1Δ and slx8Δ mutant cells. Both single mutants exhibited accumulation of SUMO foci (Fig. 6e), with an additive effect in the slx8Δ cmr1Δ double mutant. The increase in SUMO foci in these mutants correlated with the accumulation of high-molecular-weight SUMO-conjugated proteins and this accumulation was more pronounced in the cmr1Δ slx8Δ double mutant (Fig. 6f). Rather than being due to increased spontaneous genome instability (Supplementary Fig. 6e), the accumulation of high-molecular-weight SUMO conjugates in the cmr1Δ mutant probably reflects a defect in the turnover of the conjugates themselves. Importantly, Sxl8 and Cmr1 co-localized with SUMO foci (Fig. 6g), and in the absence of a functional SUMO-conjugating enzyme (ubc9Δ), INQ could still form (Cmr1 foci), but SUMO foci were abrogated (Fig. 6h). Altogether, these results suggest that INQ contains sumoylated proteins, and that Cmr1 together with Sxl8 facilitate turnover of sumoylated proteins at INQ by the proteasome, molecular chaperones or other mechanisms.

**WDR76 is the orthologue of Cmr1 in higher eukaryotes.** WDR76 is the closest orthologue of Cmr1 in higher eukaryotes (Fig. 7a). To evaluate whether the functional characteristics of Cmr1 are conserved in higher eukaryotes, we first identified WDR76 interaction partners by SILAC-based MS analysis. Consistent with data for Cmr1, the top hits of the analysis included subunits of the CCT/TRiC chaperonin (Fig. 7b and Supplementary Table 4). Moreover, two chromatin-related proteins, SUG1 and HELLS, were identified as WDR76 interactors. In addition, WDR76 was moderately enriched together with SUG1 and HELLS in nascent chromatin at replication forks in a large-scale proteomic study, suggesting that, similar to Cmr1, WDR76 might have chromatin- and replication-associated functions.

Next we investigated the WDR76 localization using a GFP-WDR76-expressing plasmid. WDR76 associated with chromatin in untreated cells and relocalized into nuclear foci under replication stress conditions (1.5 mM MMS) and after proteasome inhibition (10 μM MG132; Fig. 7c). Consistent with data from yeast, WDR76 did not co-localize with 3BP1 foci, excluding that WDR76 accumulates at the site of DSBs. Moreover, although WDR76 could be detected at some PCNA replication foci, the WDR76 foci did generally not co-localize with replication sites (Fig. 7d), consistent with the observation that Cmr1 is not a constitutive fork component. Taken together, these data suggest a structural and functional conservation of Cmr1/WDR76 in eukaryotes.

**Discussion**

Here we characterize a novel stress-induced structure, INQ, which is defined by perinuclear foci of Cmr1 and 27 other yeast proteins, and induced by genotoxic stress and proteasome inhibition. Furthermore, we provide a characterization of the role of Cmr1 in maintenance of genome integrity. Deletion of CMR1 causes increased chromosome loss and mutation rates, a defect in DNA-damage checkpoint adaptation, and accumulation of sumoylated proteins at INQ. Epistasis analyses for sensitivity to genotoxic stress place CMR1 upstream of or in parallel to MRC1, CTF18 and PPH3 in a recombination- and post-replicative repair-

**Figure 5 | Cmr1 is involved in the response to replication stress.** (a) Mrc1 and Cmr1 foci co-localize during replication stress. Co-localization between Mrc1-YFP and Mrc1-CFP was assessed in untreated cells (IG160-4A) and after treatment with MMS for 2 h. Representative images are shown. Scale bar, 2 μm. Arrowhead indicates INQ focus. (b) Quantification of co-localization of Mrc1 and Cmr1 in response to HU, MMS and MG132 (>200). (c) Point-defective Mrc1AQ protein accumulates in the nucleus. Mrc1-YFP (IG147) and Mrc1AQ-YFP (IG315) were imaged. Scale bars, 2 μm. (d) Quantification of Mrc1 AQ protein levels. Images from c were quantified (n>150). The box plot displays fluorescence intensities in arbitrary units (AU), where the area across the box identifies the mean sample value, the ends of the box are the 25th and 75th percentiles, and whiskers represent minimum and maximum values. (e) Point-defective Mrc1AQ exhibits reduced recruitment to INQ. Percentage of cells with Mrc1-AQ foci was quantified after treatment with 0.05% MMS for 2 h. Error bars represent 95% confidence intervals (n>150). (f) Mrc1 focus formation requires BTK2 and HSP42. Mrc1-YFP localization was assessed in wild-type (IG147), btk2Δ (CC1-3B) and hsp42Δ (CC2-6B) cells. Representative images of Mrc1 localization are shown. Scale bars, 2 μm. (g) Quantification of Mrc1 foci in hsp42A and btk2Δ mutants shown in f. Error bars represent 95% confidence intervals. Two replicates, n>250. (h) Schematic representation of Mrc1 fusion to a fluorescent timer. The ratio between the sfGFP (fast maturing) and mCherry (slow maturing) fluorescence intensities was calculated as a measure of Mrc1 protein turnover. (i) Quantification of Mrc1 protein turnover. The fold difference between the GFP and mCherry nuclear fluorescence was measured in wild-type (CC99B) and btk2Δ (CC102-9C) strains. Box plots were displayed as in d. Two replicates, n>100. (j) CMR1 deletion suppresses the DNA damage sensitivity of checkpoint mutants. Tenfold serial dilutions were plated on YPD or YPD containing the indicated drug. Strains were ML8-9A (wt), DP1 (cmr1Δ), IG156-7D (mrc1Δ), IG156-6C (mrc1Δ cmr1Δ), IG257-9C (pph3Δ), IG257-2C (pph3Δ cmr1Δ), IG177-9C (ctf18Δ) and IG177-8C (ctf18Δ cmr1Δ). (k) cmr1Δ is synthetic sick with homologous recombination mutants. Tenfold serial dilutions were plated on YPD or YPD containing 0.001% MMS. Strains were ML8-9A (wt), DP1 (cmr1Δ), IG164-1D (mre11Δ), IG164-28 (mre11Δ cmr1Δ), IG162-1D (rad52Δ1) and IG162-2D (rad52Δ1 cmr1Δ).
independent pathway for genotoxic stress tolerance. Moreover, the negative genetic interaction of cmr1Δ with slx8Δ is indicative of a role of Cmr1 in recycling or degrading sumoylated proteins either directly or indirectly by promoting DNA repair. The lack of DNA damage sensitivity of the cmr1Δ mutant and the wild-type levels of spontaneous Rad52 and Mec1 foci in the untreated condition supports a more direct role of Cmr1 in promoting the desumoylation or degradation of sumoylated proteins.
Importantly, many of the phenotypes of cmr1Δ are also observed on btn2Δ and/or hsp42Δ mutation, suggesting that shuttling through INQ constitutes an important aspect of Cmr1 function. However, it remains to be established whether the observed phenotypes of the btn2Δ and hsp42Δ mutants are directly related to a failure of INQ to form.

The genetic and physical interactions reported for Cmr1 in this study are largely consistent with previous studies, with the...
addition of the positive genetic interactions that we report here\textsuperscript{1,13}. With regard to other studies of nuclear foci, we acknowledge that in addition to the 27 Cmr1-co-localizing proteins reported here, other proteins are likely to localize to INQ, given the limited overlap (Apc4, Tub1, Apj1, Hos2 and Dus3) between our genome-wide screen of the GFP strain collection using MG132 and a previous screen of the same collection for nuclear foci induced by MMS, which identified 28 proteins\textsuperscript{13}. Some of the factors that form nuclear foci after MMS treatment are DNA repair proteins, which we show do not co-localize with INQ. Moreover, MMS-induced Hsp104, Mkt1, Ylr126c and Gln1 foci have been annotated as cytosolic in the previous study\textsuperscript{13}, although we find that a subset of these foci are in fact nuclear and co-localize with Cmr1.

Importantly, although Cmr1 and VHL foci co-localize in the nucleus, they exhibit different genetic requirements. Although Cmr1 foci require both BTN2 and HSP42, the relocalization of misfolded VHL to INQ only depends on BTN2 (Supplementary Fig. 4b). Interestingly, VHL foci can also be induced by MMS, although less efficiently, and these foci are exclusively nuclear and require both BTN2 and HSP42. These observations indicate that the stress caused by MMS is primarily nuclear and point to functional differences between Btn2 and Hsp42, depending on the type of stress. In particular, being a component of INQ, Btn2 is likely to be structurally involved in the formation of this nuclear compartment, whereas Hsp42 might regulate the relocalization of INQ substrates indirectly, particularly in response to replication stress. Altogether, the differences in genetic requirements and the variety of functions among its components define INQ as a multifunctional compartment gathering different kinds of substrates, only a proportion of which are misfolded proteins. The possible existence of different types of Cmr1 foci is also indicated by a subset of Cmr1 foci lacking Mrc1 (Fig. 5b). To determine the full spectrum of biological processes involving INQ, we believe that it will be important to examine protein relocalization to INQ in other stress conditions such as ultraviolet irradiation, heat shock and nutrient starvation.

As reported previously\textsuperscript{11,12}, we find that Cmr1 is a constitutive component of chromatin and interacts with DNA replication factors such as the MCM helicase and subunits of the replication factor C clamp loader (Fig. 4 and Supplementary Fig. 5). We believe these interactions to be transient, induced by replication stress and possibly mediated by posttranslational modifications. Moreover, given that Cmr1 has been shown to bind to DNA \textit{in vitro}\textsuperscript{12} and co-purifies with histones\textsuperscript{13}, it is conceivable that the association with chromatin could be achieved by direct physical interaction with DNA or nucleosomes, probably through the WD40 domain. Consistently, chromatin immunoprecipitation of Cmr1 and the replicative polymerase (Pol2) showed that Cmr1 binds chromatin independently of DNA replication (Supplementary Fig. 8a–c). In line with a recent view on the regulation of the removal and turnover of sumoylated protein complexes by proteasome-dependent degradation pathways\textsuperscript{43}, our data raise the possibility of Cmr1 being readily available on chromatin, to promote turnover of phosphorylated, ubiquitylated or sumoylated targets from stalled replication forks, thereby facilitating an efficient response to replication stress (Fig. 7e). Based on our model, this process involves INQ, in line with our establishment of a functional relationship between the ability of Cmr1 to accumulate at INQ and its role in genome maintenance. This relationship does not seem to be restricted to Cmr1 as documented by the similar accumulation of Mrc1 at INQ. In the case of Mrc1, relocalization to INQ requires Dia2, indicating that Mrc1 ubiquitylation is required. Consistently, we observed a correlation between Mrc1 protein levels and its relocalization to INQ in \textit{mrc1Δ} and \textit{btn2Δ} mutants (Fig. 5c–g), respectively, which is consistent with the reduced Mrc1 protein turnover observed when INQ is abolished by deletion of BTN2 (Fig. 5h,i).

As exemplified by Mrc1, we hypothesize that each INQ-targeted substrate will require specific mediators for their relocalization. Moreover, the accumulation of proteins at INQ on inhibition or mutation of the proteasome and/or the Skx5–Skx8 STUbL indicates that some INQ-targeted proteins are substrates of SUMO-dependent degradation.

Previous studies have described several classes of stress-induced cytoplasmic foci for protein aggregation including the JUNQ compartment and the IPOD compartment (for review see refs 42,43). JUNQ is formed by misfolded proteins, which are normally degraded in a manner dependent on chaperones and the ubiquitin proteasome system\textsuperscript{44–46}. In this study, we present evidence that a JUNQ-like structure (INQ) can form in the nucleus in response to DNA replication stress and proteasome inhibition. This conclusion is based on several lines of evidence. First, INQ localizes inside the nucleus, using as reference two independent markers of the nuclear periphery, Nup49 and Hmg1, where Hmg1 localizes continuously throughout the nuclear envelope (Supplementary Fig. 2a). Second, >95% of nuclear foci of misfolded VHL co-localized with Cmr1, while none of the juxtanuclear or cytoplasmic VHL foci contain Cmr1 (Fig. 3d,e). Third, MMS induced exclusively nuclear VHL foci, suggesting that in response to DNA replication stress VHL is recruited to INQ. Finally, the majority of the INQ-localized proteins are exclusively nuclear (67%, 18/27) in the absence of stress. We propose that the remaining proteins that do not generally localize to the nucleus in the absence of stress might be translocated to the nucleus specifically during stress, or that a minor nuclear pool of these proteins could become detectable on relocalization to INQ due to the increased local concentration.

Similar to Cmr1, its closest human orthologue WDR76 relocalizes from a diffuse nuclear distribution to distinct subnuclear foci in response to MMS and MG132. Moreover, WDR76 interacts with chromatin components and the CCT chaperonin. Although we have not further investigated the nature of WDR76 foci, the composition and properties of these structures raise the possibility of a functional similarity between INQ in yeast and promyelocytic leukemia (PML) nuclear bodies in higher eukaryotes. In particular, promyelocytic leukemia nuclear bodies are induced in number and size by genotoxic stress and proteasome inhibition\textsuperscript{44,45}; they contain both poly-sumoylated species and the RNF4 STUbL\textsuperscript{46}, and they appear to play a role in chromatin-associated processes\textsuperscript{47}. Whether WDR76 directly participates in the replication stress response remains to be addressed.

Finally, the diverse set of proteins that localize to INQ suggests that other biological processes may be regulated through this structure. For example, several components of the anaphase-promoting complex (Cdc20, Cdc27 and Apc4), which targets substrates for proteasomal degradation during the metaphase-to-anaphase transition (reviewed in ref. 48), localize to INQ, suggesting that cell cycle progression during mitosis may also require shuttling of key factors through INQ. Some INQ-localizing factors such as Gln1 and Dus3 have no reported link to maintenance of genome integrity. However, Gln1 function is linked to nutrient starvation and both proteins were reported to provide resistance to osmotic stress, suggesting that different stress responses could be coordinated at INQ or use similar signalling mechanisms\textsuperscript{49–52}. Future studies will be aimed at dissecting the mechanisms that promote relocalization and, possibly, turnover of individual proteins through INQ.

**Methods**

**Yeast strains and cell culture.** Standard media were used throughout this study\textsuperscript{53}. Standard genetic techniques were used to manipulate yeast strains\textsuperscript{53}. Unless
otherwise stated, yeasts used in this study are RADS derivatives of W303 (Supplementary Table 5). Strains from the GFP-fusion library (Invitrogen), gene disruption collection (Invitrogen) and VN-fusion collection (Bioneer) are derivatives of S288C. Exceptions and mixed backgrounds are indicated.

**Yeast constructs and plasmids.** Construction of fluorescingly tagged proteins was performed using adapter-mediated PCR. To obtain the CRM1-YCmx6 construct, a PCR product containing YCmx6 was amplified from pFA6a-VC155 (Bioneer), using CRM1-VC155-fw and CRM1-VC155-rv primers adapted with overhangs, to target integration of the construct at the C-terminal end of CRM1. The PCR product was transformed into ML8-9A-0.5% acetic acid. Immediately before MS analysis, peptides were eluted from StageTips using the EASY-nLC HPLC (Thermo Scientific) and chromatography–MS/MS using the Velos ion trap mass spectrometer (Thermo Scientific) and quadrupole Orbitrap mass spectrometers (LTQ-Orbitrap Velos or Q-Exactive, Thermo Scientific). Samples were loaded on 15 cm long reversed-phase columns (capillary column; C18-AQ, 75 μm i.d. × 30 cm) packed with 50 μm silica beads. After the initial wash buffer was pumped through the column, the first 15% of the wash buffer was eluted by vacuum centrifugation and the samples were acidified with 2 ml 60% acetonitrile, 0.5% acetic acid, sample volume was reduced to ~1 μl by vacuum centrifugation and the samples were acidified with 2 µl 6M HCl. In-gel trypsin digestion was carried out overnight at 37 °C, peptides were extracted by incubating the gel pieces with 10 % (v/v) acetonitrile, 0.1 % (v/v) TFA, peptides were desalted and lyophilized. The analysis was performed using liquid chromatography–MS/MS with the LTQ-Orbitrap Velos and Eksigent nanoLC mass spectrometers operated in positive ion mode, data-dependent manner, automatically switching between MS and MS/MS acquisition. The survey full scan was set to scan m/z 200–1,700 for the Velos and 400–2,000 for the LTQ-Orbitrap Velos. For the LTQ-Orbitrap Velos, ions were fragmented with normalized collision energy of 30,000 and 70,000 for the Velos and Q Exactive, respectively. The top 10 or 12 most intense ions were sequentially isolated and fragmented by higher-energy C-trap dissociation. An ion selection threshold of 5,000 was used. Peptides with unspecified charge states, as well as with charge state < +2 were excluded. Peptides were matched against the Saccharomyces Genome Database release 63 containing 6,717 putative protein coding genes (http://genome.yeastgenome.org). The search engine X!TANDEM was used for the software, except that ‘Minimum Ratio Count’ was set to 1. Cysteine carboxymethylation was searched as a fixed modification, whereas protein
N-terminal acetylation and methionine oxidation were searched as variable modifications. Database search was performed with a mass tolerance of 6 p.p.m. for precursor ions and 20 p.p.m. for fragment ions. False discovery rate was estimated using a target-decoy approach, allowing a maximum of 1% false identifications from the reversed-sequence database.

For determining WDR76 interactome, HeLa cells were cultured in DMEM medium supplemented with 10% fetal bovine serum, L-glutamine, penicillin and streptomycin. For the pull-down interactions, cells were cultured in 10 cm Petri dishes. One mg WDR76 (Sigma) was incubated with 84 µg ml−1 l-arginine and 146 µg ml−1 l-lysine or 84 µg ml−1 l-arginine, 150 mM NaCl, 1% NP-40, 0.1% Na-deoxycholate, 1 mM EDTA and protease inhibitors 5 mM β-glycerophosphate, 5 mM NaF, 1 mM Na-orthovanadate, complete protease inhibitor cocktail (Roche) after 48 h. Lysates were cleared by centrifugation at 17,000g for 15 min at 4°C, and GFP-WDR76 and its interacting proteins were enriched using GFP-Trap resin (ChromoTek) for 2 h. Proteins were resolved by SDS-PAGE and digested in gel. Peptide fractions were assayed on a quadrupole Orbitrap mass spectrometer (Q-Exactive, Thermo Scientific) equipped with a nanoflow HPLC system (Thermo Scientific) by LC-MS/MS analysis. Raw data files were analysed using MaxQuant software (version 1.2.2.9)56.

Synthetic genetic array. SGA technology was used to transfer the cmr1::kanMX and the cmr1-YFP::NatMX NLS-yEmRFP::URA3 constructs from the query strains (IG241 and IG189-10B, respectively) to each of the VN fusion library (Bioneer) and gene deletion collection (Invitrogen) strains, respectively. For analysis of cmr1::kanMX interactions, SGA analysis was performed in quadruplicates (1,536 format). MATa meiotic progeny derived from the cmr1::kanMX query strain (IG105) or the control strain (SG936) were tested for viability on YPD plates containing 200 mM HU. Plates were scanned (ScanMaker 70, Scanalytica) and analysed using the Spot software (Gabor Technologies). DNA damage sensitivity and mutation rates.

For determining chromosome loss, the cdc13-1 mutant was introduced into relevant mutant strains by genetic crossing. To measure checkpoint adaptation61, cells were grown shaking in YPD to mid-log phase, shifted to 32°C for 2 h, sonicated, diluted appropriately and spread onto preheated YPD plates at 32°C. After incubation of the plates at 32°C for 24 h, images were acquired of 200–300 microcolonies and the number of cell bodies in each colony counted.

Checkpoint adaptation assay. The cdc13-1 mutation was introduced into relevant mutant strain by genetic crossing. To measure checkpoint adaptation61, cells were grown shaking in YPD to mid-log phase, shifted to 32°C for 2 h, sonicated, diluted appropriately and spread onto preheated YPD plates at 32°C. After incubation of the plates at 32°C for 24 h, images were acquired of 200–300 microcolonies and the number of cell bodies in each colony counted.

Microscopy of human cells. Cells were grown as described66. Images were collected using a DeltaVision system.

DNA damage sensitivity. For analysis of drug sensitivity on solid medium, tenfold serial dilutions were prepared from a saturated overnight culture. Zeocin (Invitrogen), MMS (Sigma), HU (Sigma), 4-nitroquinoline-1-oxide (Sigma) and camptothecin (Sigma) were added to the final concentrations stated in the figure legends. After spotting, the plates were incubated at 30°C for 2–4 days.

Protein analysis. For detection of Cmr1 protein levels, whole-cell extracts were obtained by Sigma Bio-Clean Protocol. Cells were washed twice in 1X PBS and with 1% Triton X-100 to lyse the cells. DNA was isolated by shaking with SDS and phenol/chloroform extraction, followed by sodium acetate precipitation and ethanol precipitation. DNA was dissolved in water and quantified by NanoDrop.

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**Author contributions**

I.G., C. Colding, S.S. and M.L. conducted the majority of the yeast experiments. P.H., P.B. and C. Choudhary performed MS-based protein–protein interaction analysis. K.N. and A.G. conducted microscopy of human cells. J.O. and E.H. performed the yeast screen for mutants that affect mutation rates. D.P.M. constructed strains. All authors contributed to designing experiments and writing the manuscript.

**Additional information**

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