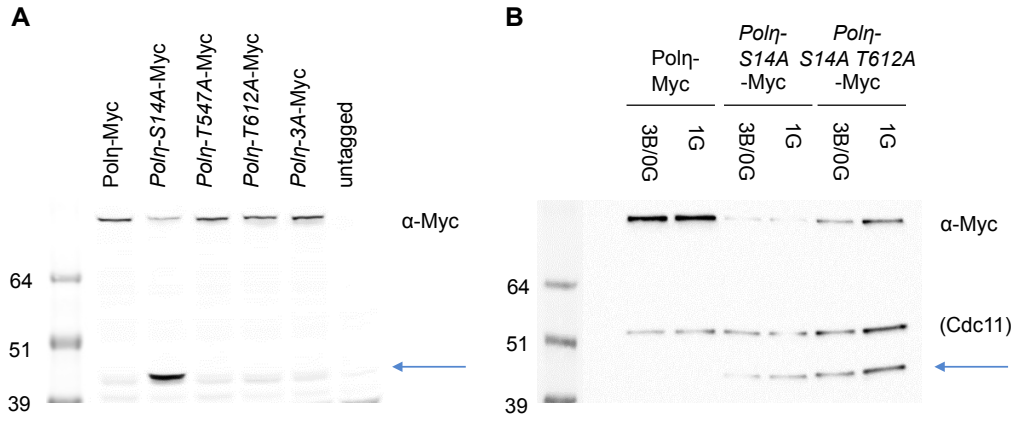
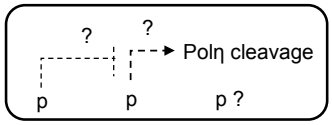



C




| | S14 | T547 | T612 | ~ 40 kD cleavage product |
|---|---|---|---|---|
| WT | p | p | p | no |
| *Polη-S14A* | x | p | p | yes |
| *Polη-T612A* | p | p | x | no |
| *Polη-T547A* | p | x | p | no |
| *Polη-S14A T612A* | x | p | x | yes |
| *Polη-3A* | x | x | x | no |

**Figure S1.** A population of Polη is being cleaved in *Polη-S14A* and *Polη-S14A T612A* mutants. (A) Protein levels of Myc-tagged Polη-phosphorylation site mutants, during $G_2$/M arrest. The Western blot here is the same as the one in Figure 2A, showing the α-Myc bands from 39 kDa and above. (B) Protein levels of Myc-tagged *Polη-S14A* single and -*S14A T612A* double mutants. Cdc11 was used as loading control. Blue arrows in (A) and (B) indicate the cleavage product. (C) Protein stability of the Polη-phosphorylation mutants in summary, together with the proposed actions of Polη-S14 and T547 phosphorylation shown in the box. 3B/0G, three hours benomyl/zero hour time-point of *GAL*-induction; 1G, one hour *GAL*-induction; p, phosphorylated; x, mutated residue(s).

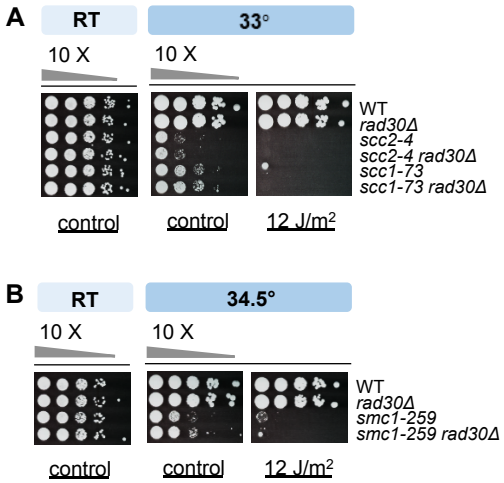



**Figure S2.** UV sensitivities of cohesin mutants at restrictive temperature. (A-B) Each strain was grown to mid-log phase and the following 10-fold serial dilutions were spotted on YEPD, with or without follow-on UVC exposures. Scc2 and Scc1 are inactivated at 33°; Smc1 is inactivated at 34.5°. All plates were documented on the third day. RT, room temperature. One representative experiment from two independent spot assays performed is shown.

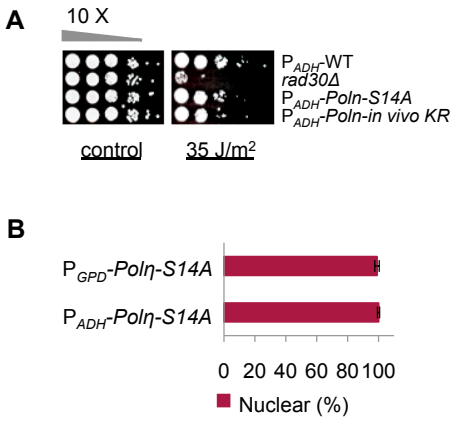



**Figure S3.** UV sensitivities and nuclear accumulation of selected Polη point mutants controlled by the constitutive strong *ADH* promoter. (A) UV spot assay of P$_{ADH}$-*Polη-S14A* and P$_{ADH}$-*Polη-in vivo KR*. Each strain was grown to mid-log phase. Ten-fold serial dilutions were then spotted on YEPD plates and exposed to 35 J/m$^2$ UVC. Plates were documented after three days at room temperature. (B) Quantitation of *Polη-S14A* nuclear accumulation. In situ staining was performed, the percent of cells displaying nuclear accumulation was determined as the cells positive for anti-Myc, with overlapping anti-Myc and DAPI signals. *Polη-S14A*-Myc controlled by the alternative constitutive strong promoter *GPD* was used for comparison. Means ± STDEV from at least two independent experiments are shown. YEPD, YEP media supplemented with glucose.

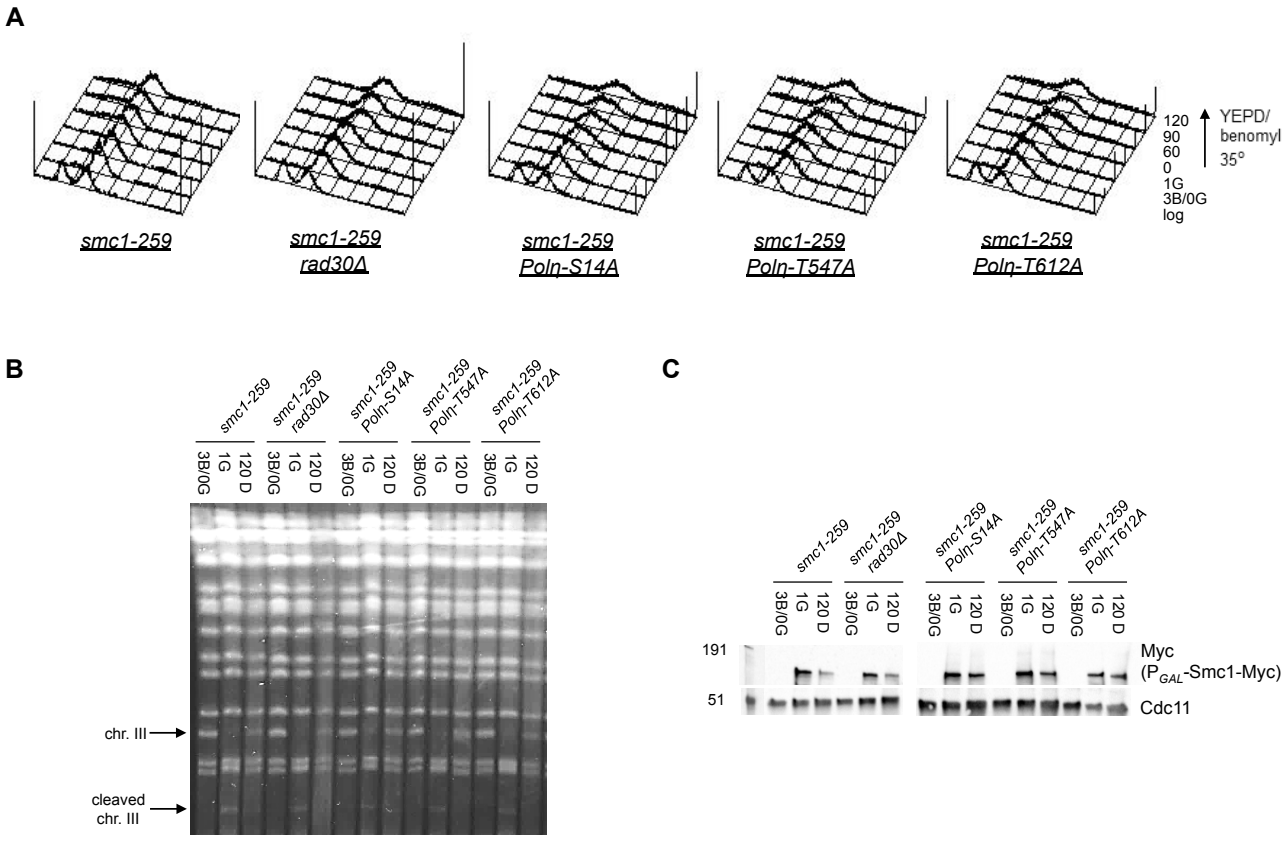



**Figure S4.** Examples of included controls for a typical damage-induced cohesion experiment. Shown here are the controls for the damage-induced cohesion experiment of the single Polη-phosphorylation mutants in Figure 6C. (A) FACS analysis to confirm benomyl induced $G_2$/M arrest. (B) Pulsed-field gel electrophoresis to monitor efficiency of break induction on Chr. III ($P_{GAL}$-*HO*). (C) Western blot to monitor expression of ectopic $P_{GAL}$-*SMC1-MYC* (Smc1 WT). Cdc11 was used as loading control. 3B/0G, three hours benomyl/zero hour time-point of *GAL*-induction; 1G, one hour *GAL*-induction; 120 D, 120 minutes in YEP media supplemented with glucose at 35°; Chr., chromosome; HO, Homothallic switching endonuclease.

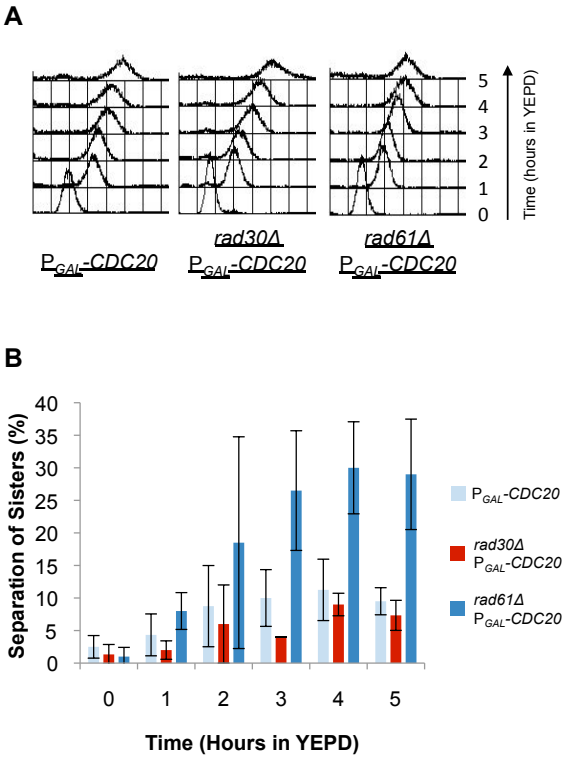



**Figure S5.** Sister chromatid cohesion maintenance is independent of Polη. (A) FACS analysis to monitor cell cycle distribution. Cells were initially synchronized in $G_1$ by α-factor in YEPG, and subsequently released into YEPD for arrest in $G_2$/M by inactivating *CDC20* expression. (B) Sister chromatids separation was monitored at the *URA3* locus on Chr. V by the Tet-O/TetR-GFP system (see materials and methods for details). Means ± STDEV from at least two independent experiments are shown. YEPG, YEP media supplemented with galactose; YEPD, YEP media supplemented with glucose; Chr., chromosome.