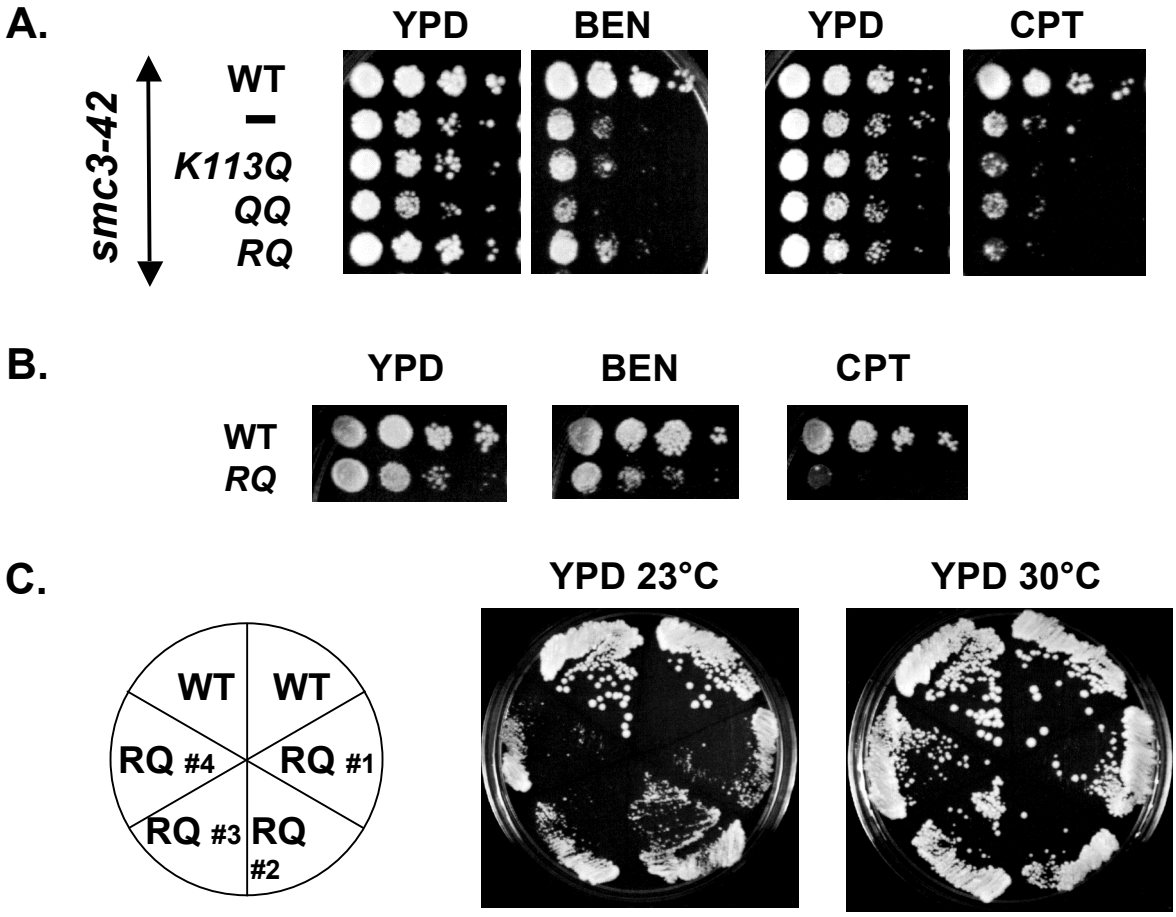
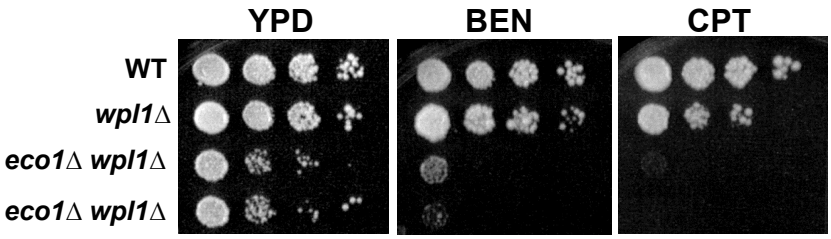
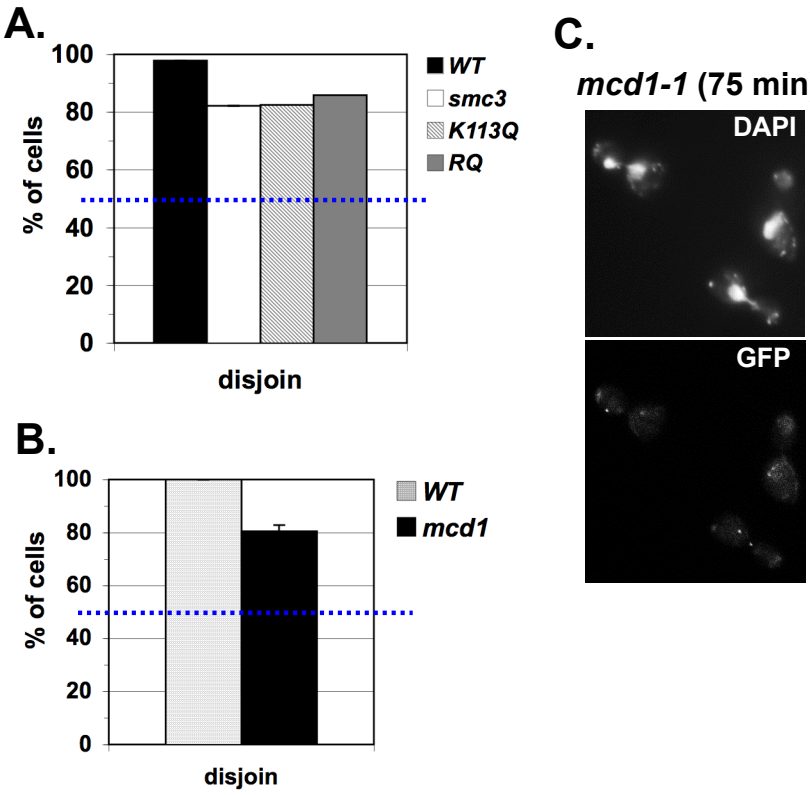


Supplemental Figure 1. *smc3* acetyl-mimics are sensitive to benomyl, campotothecin and are cold-sensitive. (A) Acetyl-mimics in an *smc3-42* background. Strains from figure 1A grown and plated as described in figure 1A. Parent *smc3-42* alone (-) or containing a second *SMC3* allele (WT), (*K113Q*), (*QQ*) or (*RQ*) were plated on YPD alone or containing 10ug/ml benomyl (BEN) or and incubated at 30°C for 3d or 5d, respectively (left side), or YPD alone or containing 10ug/ml camptothecin (CPT) and incubated at 23°C for 3d or 5d, respectively (right side). (B) & (C) Haploids from figure 1B containing either *SMC3* (WT) or *smc3-RQ* (*RQ*) as the sole *SMC3*. (B) Cells were grown in YPD liquid at 30°C, plated in 10 fold dilutions on YPD alone, or containing 10ug/ml benomyl (BEN) or 10ug/ml Camptothecin (CPT) then incubated at 30°C for 3d. (C) Cells were dilution streaked onto YPD and incubated at 23°C for 4d or 30°C for 3d.



Supplemental Figure 2. *eco1*Δ *wapl1*Δ cells are sensitive to microtubule and DNA damaging agents. WT, *wpl1*Δ and *eco1*Δ *wpl1*Δ haploids from Figure 3A were grown in YPD liquid (23°C), plated in 10 fold serial dilutions onto YPD alone or containing 10ug/ml benomyl (BEN) or 10 ug/ml camptothecin (CPT). YPD was incubated for 3d (23°C) and BEN and CPT plates for 4d (23°C).



Supplemental Figure 3. G1 phase cells of cohesin mutants that completed one cell cycle without functional cohesin segregate sister chromatids. (A & B) G1 phase cells that completed one cell cycle at 35.5°C as described in Figure 4 and scored for disjunction. Chr. IV segregation was monitored at a *CEN4*-proximal locus (*TRP1)* marked with GFP. Dotted line marks the 50% disjunction expected for random segregation. (A) chr IV disjunction in G1 phase cells from figure 4, *smc3-42* cells alone (*smc3*) or with a second *SMC3* allele, (WT), (*K113Q*) or (*RQ*). (B & C) WT or *mcd1-1* mutant cells from figure 5. (B) chr IV disjunction in G1 phase cells (C) Micrographs of *mcd1-1* cells 75min after release from the initial G1 phase arrest.

Yeast strain table*

VG3358-3B	Mat**a** *smc3-42 LacO-NAT::lys4 LacIGFP-HIS3:his3-11,15 trp1-1 leu2-3,112 ura3-52 bar1*
VG3377-1A	same as VG3358-3B except *SMC3-URA3:ura3-52*
VG3378-2A	same as VG3358-3B except *smc3K112Q,K113Q-URA3:ura3-52*
VG3423-1A	same as VG3358-3B except *smc3K113Q-URA3:ura3-52*
VG3424-2A	same as VG3358-3B except *smc3K112R,K113Q-URA3:ura3-52*
VG3464-16C	Mat**a** *smc3Δ::HPH LacO-NAT::lys4 LacIGFP-TRP1:his3-11,15 trp1-1 leu2-3,112 ura3-52 bar1* + pEU42 (*SMC3 URA3 CEN*)
VG3471-WT	Mat**a** *smc3Δ::HPH LacO-NAT::lys4 LacIGFP-TRP1:his3-11,15 trp1-1 leu2-3,112 ura3-52 bar1* + pEU41 (*SMC3 LEU2 CEN*)
VG3471-RQ	same as VG3471-WT except + pEU41-RQ (*smc3K112R,K113Q LEU2 CEN*)
VG3357-3A	Mat**a** *smc3-42 LacO-NAT:TRP1 LacIGFP-HIS3:his3-11,15 trp1-1 leu2-3,112 ura3-52bar1*
VG3447-1B	same as VG3357-3A except *SMC3-URA3:ura3-52*
VG3449-3B	same as VG3358-3B except *smc3K113Q-URA3:ura3-52*
VG3450-4B	same as VG3358-3B except *smc3K112R,K113Q-URA3:ura3-52*
VG3460-2A	Mat**a** *LacO-NAT:TRP1 LacIGFP-HIS3:his3-11,15 trp1-1 leu2-3,112 ura3-52 bar1*
VG3456-2C	Mat**a** *mcd1-1 LacO-NAT:TRP1 LacIGFP-HIS3:his3-11,15 trp1-1 leu2-3,112 ura3-52 bar1*
VG3349-1B	Mat**a** *LacO-NAT::lys4 LacIGFP-HIS3:his3-11,15 trp1-1 leu2-3,112 ura3-52 bar1*
VG3360-3D	Mat**a** *wpl1Δ::HPH LacO-NAT::lys4 LacIGFP-HIS3:his3-11,15 trp1-1 leu2-3,112 ura3-52 bar1*
VG3503-1B	Mat**a** *wpl1Δ::HPH ecol1Δ::G418 LacO-NAT::lys4 LacIGFP-HIS3:his3-11,15 trp1-1 ura3-52 leu2-3,112 bar1*
VG3503-4A	same as VG3503-1B
VG3506-5D	Mat**a** *ctf7-203 (eco1-203) LacO-NAT:TRP1 LacIGFP-HIS3:his3-11,15 trp1-1 leu2-3,112 ura3-52 bar1*
VG3513-1B	Mat**a** *wpl1Δ::HPH LacO-NAT:TRP1 LacIGFP-HIS3:his3-11,15 trp1-1 leu2-3,112 ura3-52 bar1*
VG3502-2A	Mat**a** *wpl1Δ::HPH ecol1Δ::G418 LacO-NAT:TRP1 LacIGFP-HIS3:his3-11,15 trp1-1 ura3-52 leu2-3,112 bar1*
VG3502-4C	same as VG3502-2A

* All yeast strains are the A364A background.