

Supplemental Materials

Molecular Biology of the Cell

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SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. *WPL1*-centric model for regulation of cohesion establishment. (Left side). Wpl1p inhibiting establishment during S phase. Eco1p is inactive (grey box) so Wpl1p (black triangle & blue box) destabilizes cohesin (red oval) binding to chromosomes. (Right side). Eco1p mediated Smc3p acetylation antagonizes Wpl1p to enable establishment. Eco1p (blue box) acetylates cohesin on the Smc3p subunit. Acetylated cohesin is refractory to Wpl1p mediated destabilization of cohesin.

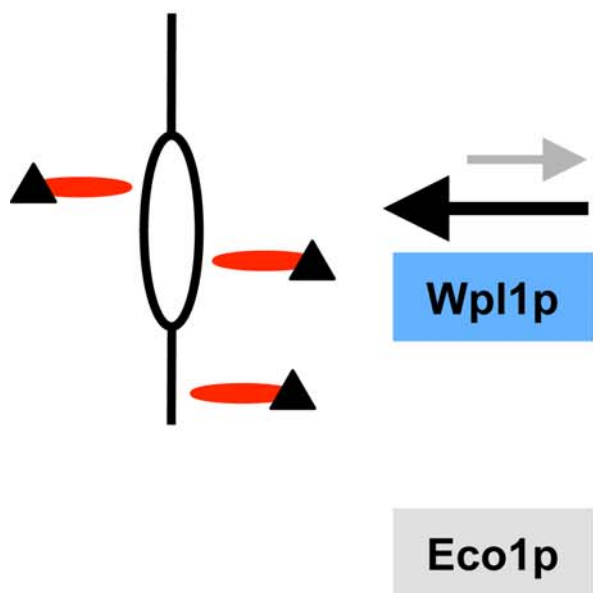
Supplemental Figure 2. *ECO1-AID wpl1Δ* and *eco1Δ wpl1Δ* cells are phenotypically similar after *ECO1-AID* depletion. (A) Dilution plating to assess affect of auxin mediated Eco1p depletion. Haploid WT (VG3349-1B), *ECO1-AID* (VG3633-2D), *eco1Δ wpl1Δ* (VG3503-4A) and *ECO1-AID wpl1Δ* (VG3687-2A) strains were grown to saturation at 23°C then plated in 10-fold serial dilutions onto YPD alone, or containing auxin (750μM), BEN (10μg/ml) or both auxin + BEN (750μM & 10μg/ml), respectively then incubated 2d at 23°C (B) Regimen for depleting *AID* tagged proteins in G1 through arrested in mid-M phase for cohesion assays. (C) Cohesion loss at a *CEN*-distal locus *LYS4* after *ECO1-AID* depletion. Haploid WT (VG3349-1B), *ECO1-AID* (VG3633-2D), *eco1Δ wpl1Δ* (VG3503-4A) and *ECO1-AID wpl1Δ* (VG3687-2A) strains were depleted for Eco1-AIDp in G1 through mid-M phase. The percentage of cells with 2-GFP spots (sister separation) is plotted. 100-200 cells scored for each data point. The lack of G1 cells with 2-GFP spots demonstrates absence of pre-existing aneuploidy. DNA content (right side).

Supplemental Figure 3. Genetic screen to identify suppressors of *eco1Δ wpl1Δ* mutant drug sensitivity. (A) Determining lethal concentrations of benomyl (BEN) and camptothecin (CPT) for *eco1Δ wpl1Δ* cells. Haploid WT (VG3349-1B), *eco1Δ wpl1Δ* (VG3503-4A) were grown to saturation at 23°C, then 10⁴ and 10⁶ cells spotted onto YPD alone or containing either BEN or CPT at 5μg/ml, 10μg/ml or 15μg/ml then incubated 3d at 23°C. (B) Schematic of the suppression screen of *eco1Δ wpl1Δ* cells. Haploid *eco1Δ wpl1Δ* cells were dilution streaked onto YPD and incubated 3d at 23°C to allow colony formation from single cells. A small amount of a single colony was inoculated into 5ml YPD and grown overnight at 23°C to saturation. ~10⁷ of saturated cells were plated onto BEN (12.5μg/ml or 15μg/ml) or CPT (12.5μg/ml or 15μg/ml) then grown at 23°C 4d to select drug resistant suppressor mutants. A different single colony from YPD plates was used for each selection trial to generate independent suppressors.

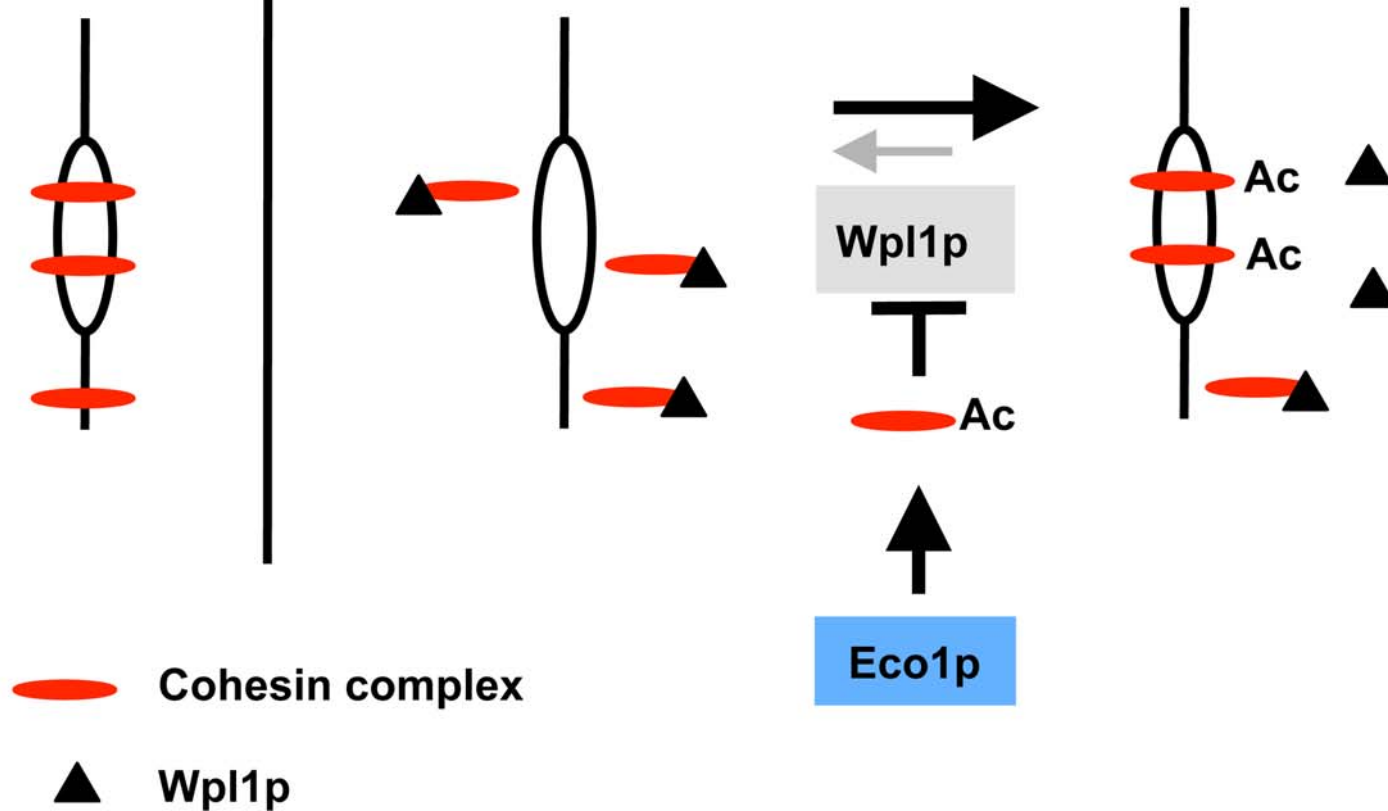
Supplemental Figure 4. *smc3-D1189H* cohesion, like WT cohesion, requires Scc2p for viability and cohesion. (A) Dilution plating to assess viability after auxin mediated Scc2p depletion. Haploid wild-type (WT; VG3593-7C), *SCC2-AID* (*SMC3 SCC2-AID*; VG3615-4C) and *smc3-D1189H SCC2-AID* (VG3616-9B) were grown and plated as described in Fig 2A onto YPD alone or containing auxin (500μM) then incubated 2d at 23°C. (B) Cohesion loss at *CEN*-distal locus *LYS4* in mid-M phase cells. Strains in A were depleted for Scc2p-AID in G1 through mid-M phase arrest as depicted in Suppl Fig 2B. The percentage of cells with 2 GFP spots (cohesion loss) is plotted. 100-300 cells were scored for each data point.

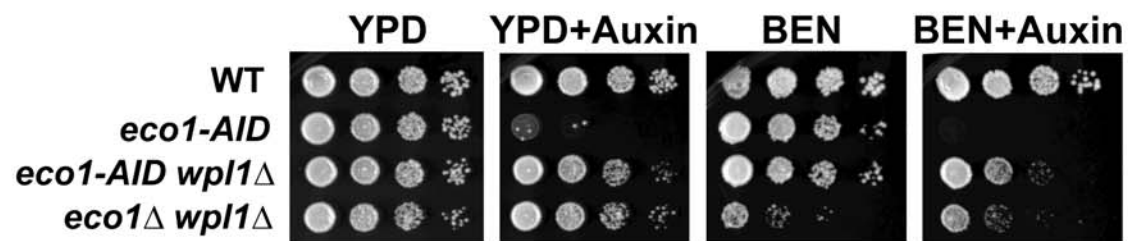
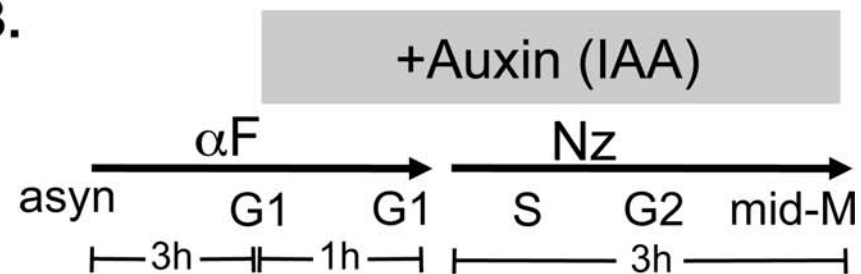
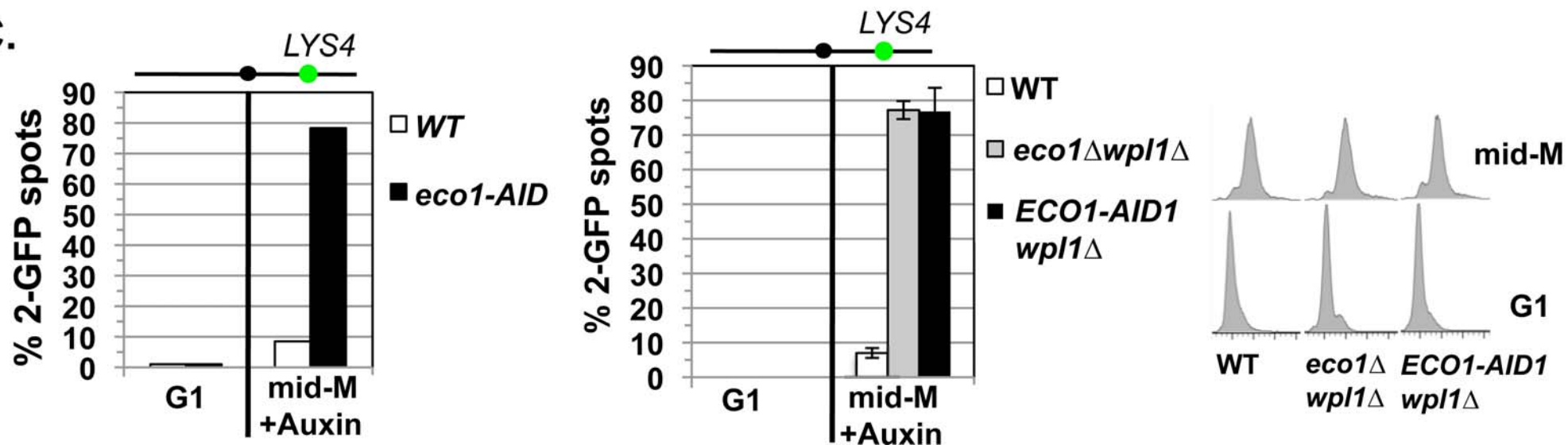
Supplemental Figure 5. Characterization of *SMC3-AID* and the *smc3-RR* allele in the *SMC3-AID* background. (A) Western Blot to assess auxin induced Smc3-AIDp depletion. Haploid *SMC3-AID* (VG3651-3D) was arrested in G1 phase (α F), auxin added to induce Smc3p-AID depletion (α F + auxin) then cells released and arrested in mid-M phase in the presence of auxin (Nz + auxin) as depicted in Suppl Figure 2B. Total protein extracts were analyzed by Western Blot to assess Smc3-3V5-AIDp presence and depletion (α V5). Tubulin (α TUB) was monitored as a loading control. (B) Cohesion defect of cells bearing *SMC3-AID* as sole Smc3p. Haploid wild-type (*SMC3*; VG3620-4C) and *SMC3-AID* (VG3651-3D) were depleted for Smc3-AIDp from G1 through mid-M phase arrest as depicted in Suppl Fig 2B. The percentage of cells with 2-GFP spots (cohesion loss) is plotted. 100-200 cells scored for each data point. (C) Assessing *smc3-RR* viability after Smc3-AIDp depletion. Haploid WT (VG3349-1B), or strains bearing *SMC3-AID* alone (VG3651-3D), or containing a second *SMC3* allele, either WT (*SMC3 SMC3-AID*; MB81-1A) or *smc3-RR* (*smc3-RR SMC3-AID*; MB79-1A) grown and plated as described in Figure 2A onto YPD alone or containing auxin (500 μ M) then incubated 2d at 23°C. (D-F) DNA content of cells analyzed for cohesion loss in figure 6. (D) Strains from Figure 6C used to assess *smc3-RR* in the *SMC3-AID* background. (E) Strains from Figure 6D assayed for *smc3-RR-D1189H* cohesion. (F) Strains from Figure 6F assessing effect of *ECO1-AID* depletion in *smc3-D1189H* cells.

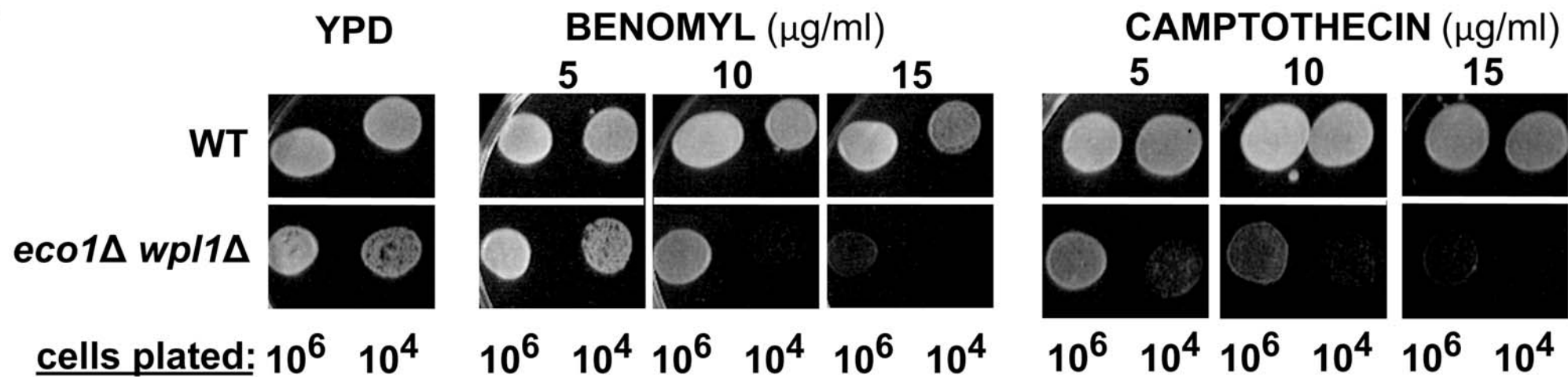
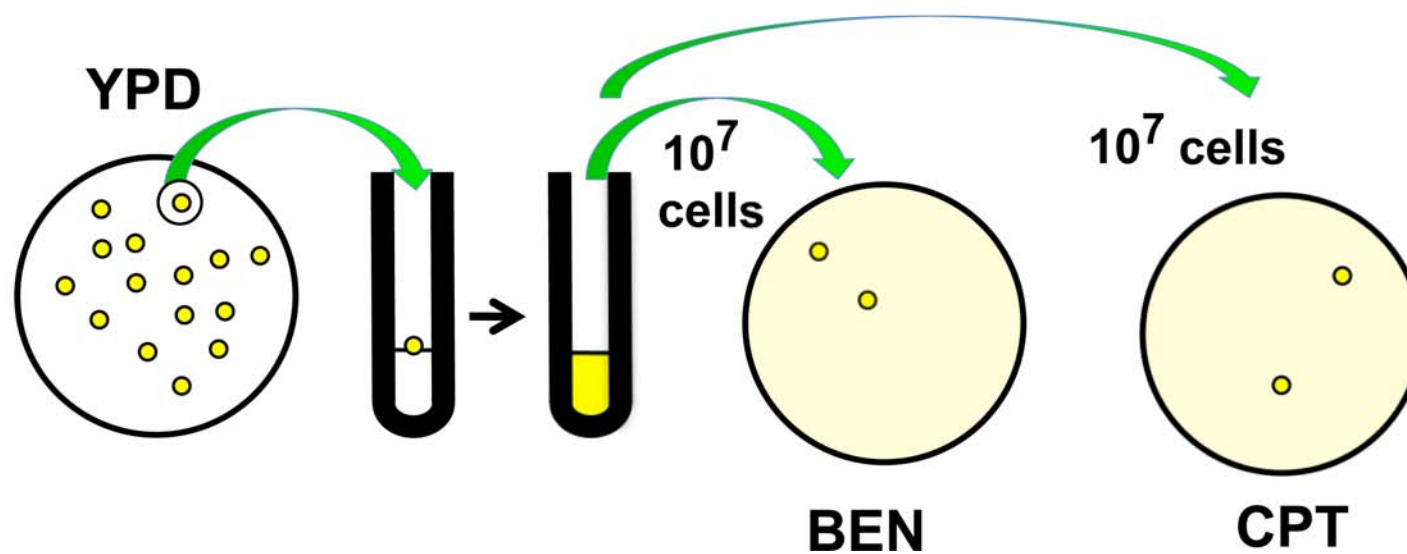
Wpl1p inhibiting establishment

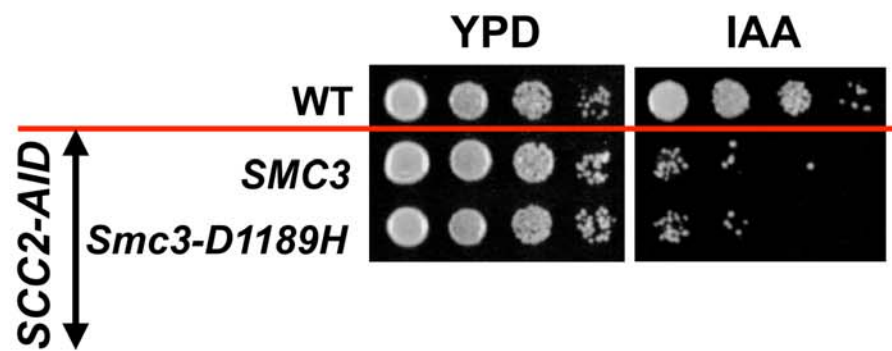


Establishment by antagonizing Wpl1p



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