



Characterizing the effect of Scc2p-AID or Scc3p-AID depletion in normal and fusion cohesin strains.

(A). Schematic of regimen used to synchronously arrest cells in mid-M phase. (B-D) Strains with normal cohesin or fusion cohesin alone or bearing Scc2p-AID or Scc3p-AID from Figure 2A were synchronously arrested in mid-M then processed to generate data presented in Figure 3A and 3C. (B) FACS to confirm arrest of cells. (C-D) Assessing depletion of Scc2p-AID and Scc3p-AID assessed. Protein extracts were made from G1 arrested cells before and after auxin treatment and from cells synchronous arrested in mid-M then subjected to SDS-PAGE and analyzed by Western Blot. 3V5-AID tagged protein depletion was monitored using mouse antibodies against V5 (αV5) and rabbit anti-tubulin for a loading control (αTUB2). (C) Scc2p-3V5-AID depletion. (D) Scc3p-3V5-AID depletion (E) Mcd1p and Smc3p-Mcd1p fusion levels in mid-M arrested cells. Protein extracts from mid-M phase cells in C & D were analyzed by Western Blot. Mcd1p and fusion Smc3-Mcd1p fusion protein levels were monitored using rabbit antibodies against Mcd1p (αMCD1) and rabbit anti-tubulin for a loading control (αTUB2).