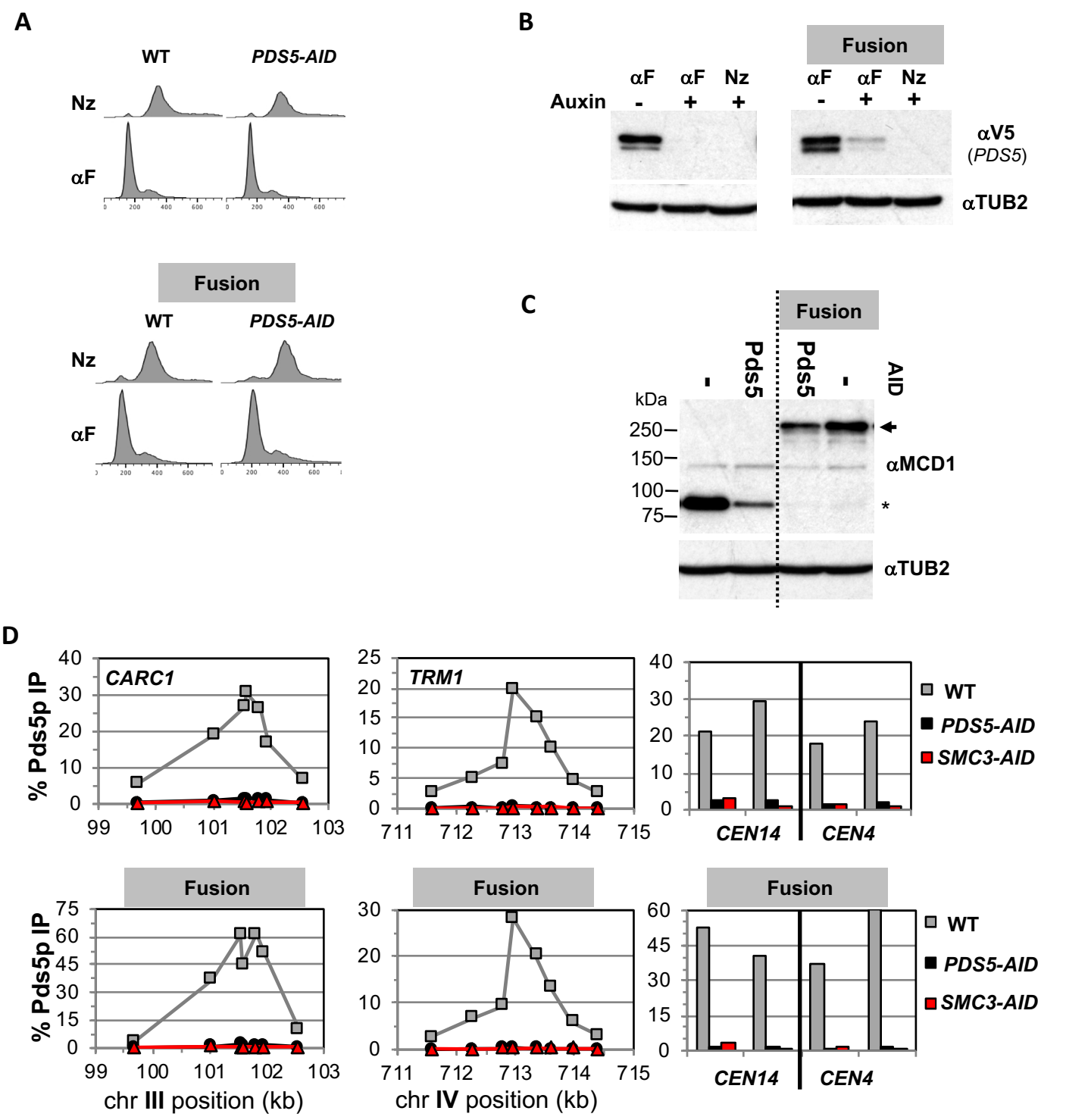

Characterizing the effects of Pds5p-AID depletion in normal and fusion cohesin strains. Strains with normal cohesin or fusion cohesin alone or bearing Pds5p-AID from Figure 2B were synchronously arrested in mid-M then processed to generate data presented in described in Figures 3B & 3D. (A) FACS to confirm arrest of cells. (B) Assessing depletion of Pds5p-AID. Protein extracts were made from G1 arrested cells before and after auxin treatment and from synchronous arrest in mid-M were subjected to SDS-PAGE and analyzed by Western Blot. (B) Pds5p-3V5-AID depletion was monitored using mouse antibodies against V5 (αV5) and rabbit anti-tubulin for a loading control (αTUB2) (C) Mcd1p and Smc3p-Mcd1p fusion levels in mid-M arrested cells. Protein extracts from mid-M phase cells in B were analyzed by Western Blot. Mcd1p and Smc3-Mcd1p fusion protein levels were monitored using rabbit anti-Mcd1p antibodies (αMCD1) and rabbit anti-tubulin for a loading control (αTUB2). (D) Pds5p binding to chromosomes using ChIP. Mid-M phase cells from B-C were fixed and processed for ChIP to assess Pds5p binding after Pds5p depletion as described in Figure 3. Pds5p binding was assayed using rabbit anti-Pds5p antibodies (αPDS5). WT (grey), *PDS5-AID* (black) and *SMC3-AID* (red)