Supplemental Materials

Molecular Biology of the Cell

Ganesan et al.

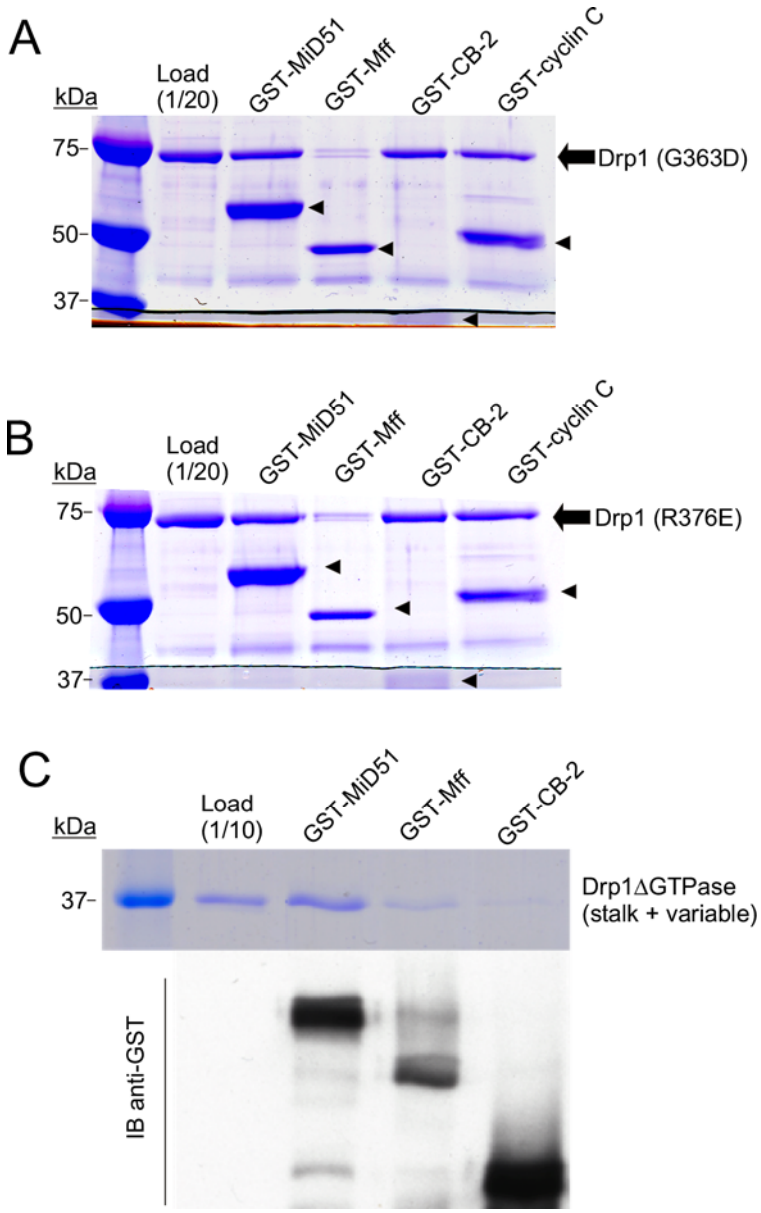

Figure S1. Cyclin C and CB2 bind Drp1 mutants defective for oligomerization. Pulldownexperiments with 1 His_6-Drp1 mutants G363D (A) and R376E (B) was performed with 1 GST-tagged MiD51(N118), Mff(TM), CB2or cyclin C. The bait proteins were recovered with glutathione beads, resolved in SDS-PAGE and stained with Coomassie. Location of the Drp1 derivatives is indicated by the arrow. The GST-fusion bait proteins are indicated by the arrowheads. Molecular weight markers (kDa) are indicated on the

left. (C) The pulldown experiments were repeated as just described with the His_6-Drp1 derivative deleted for the GTPase domain but containing the stalk and variable regions. Loading controls for the GST fusion proteins was accomplished by Western blot analysis probing for GST.

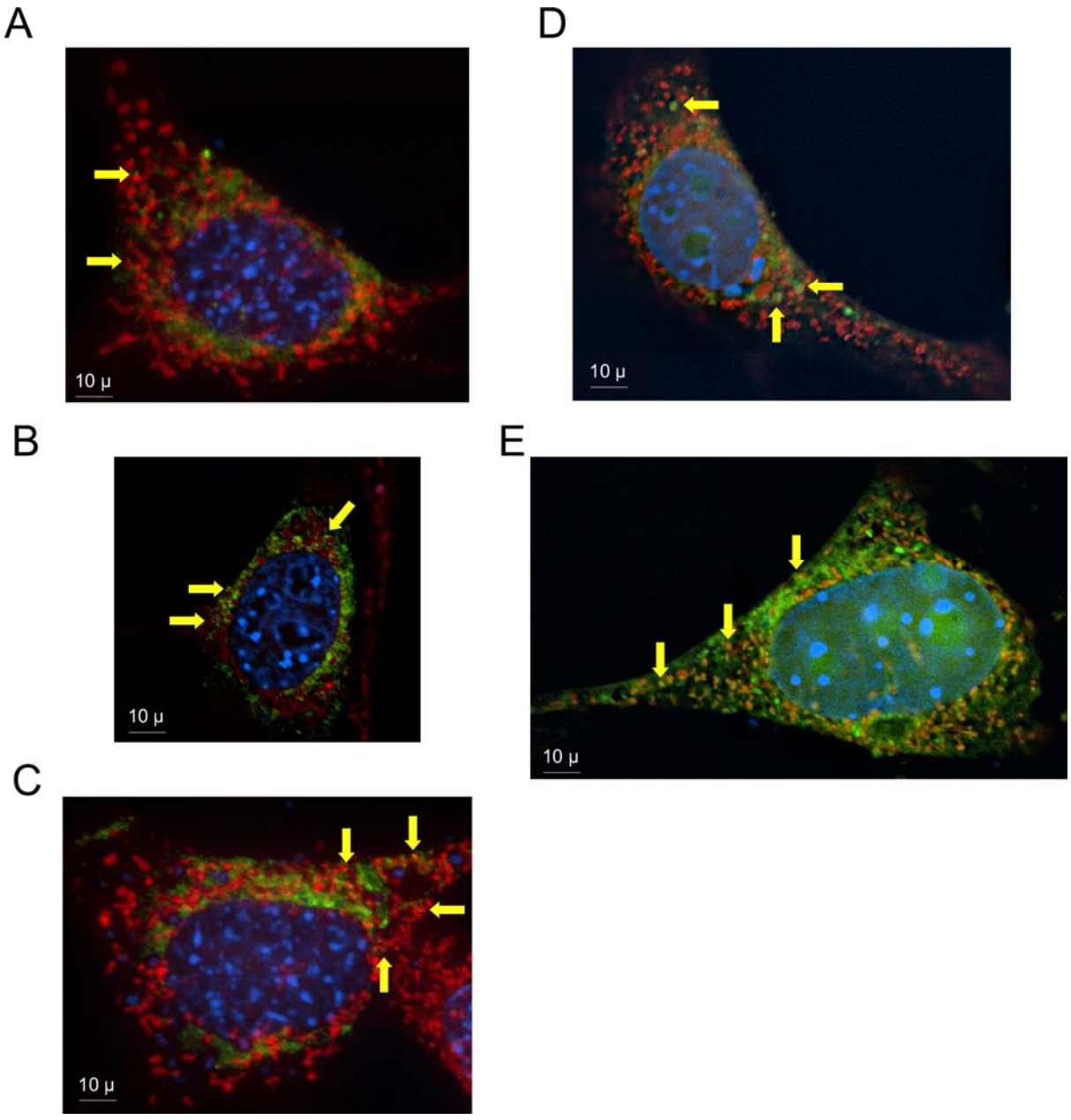

Figure S2. Mitochondria and EGFP-CB2 co-localization. (A, B, C) Non-stressed CCNC-/- MEF cultures transiently expressing EGFP-CB2 were stained with MitoTraker Red and DAPI to identify mitochondria and nuclei, respectively. Arrows indicate locations of EGFP-CB2-mitochondrial co-localization. Note the small foci on the edge of the mitochondrial signal. (D,E)

The same cells treated with cisplatin for 24 h. Note the extensive co-localization with the EGFP-CB2 signal decorating the entire fragmented mitochondria.

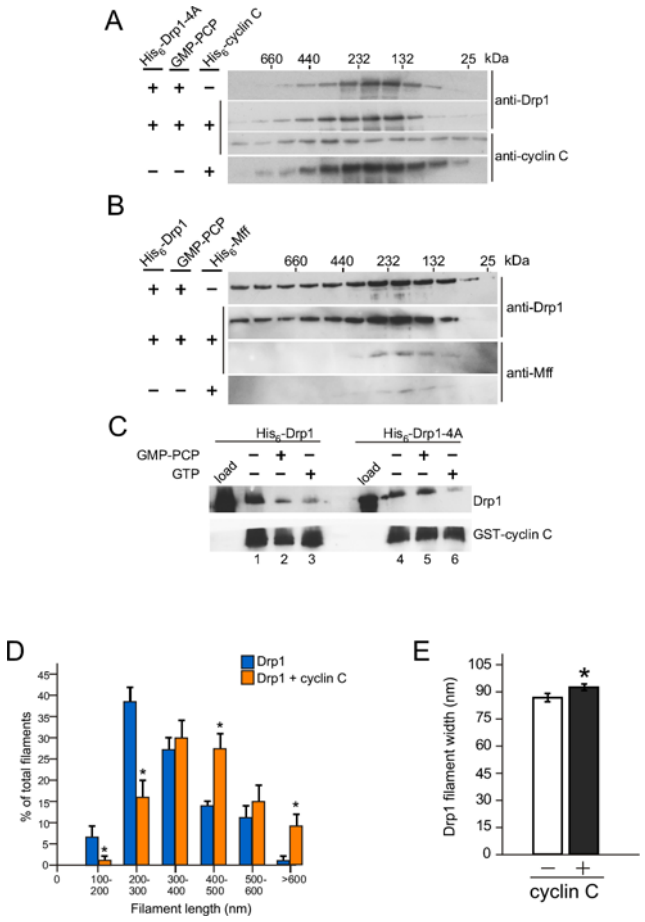

Figure S3. Cyclin C associates with aggregate-defective Drp1 mutants. (A) SEC fractions fromHis_6-Drp1-4A (1 µM) + 2mM GMP-PCP/Mg^{2+} without or with His_6-cyclin C (5 µM) were blotted and probed for Drp1 and cyclin C as indicated. Molecular weight standards are indicated on the top of the panel. (B) As in (A) except wild-type His_6-Drp1 (1 µM) and His_6-Mff (5 µM) were analyzed. (C) His_6-cyclin C was bound to His_6-Drp1 or His_6-Drp1-4A with GTP or GMP-PCP as indicated. Western blot analysis of His_6-Drp1 or His_6-Drp1-4A was used to monitor binding. The load (1/5 total added to the reaction) is provided as standard for Drp1 migration. The blot was stripped and reprobed for cyclin C to control for similar addition to the binding reactions. (D) The percentages of filaments within specific lengths were plotted for Drp1 (blue) or Drp1 + cyclin C (orange) bars. Error bars are mean +/- SEM (n = 100). Asterisks indicate $p < 0.05$. (E) The width

of Drp1 filaments was measured with and without cyclin C. Error bars are mean +/- SEM (n = 100). Asterisks indicate $p < 0.05$.

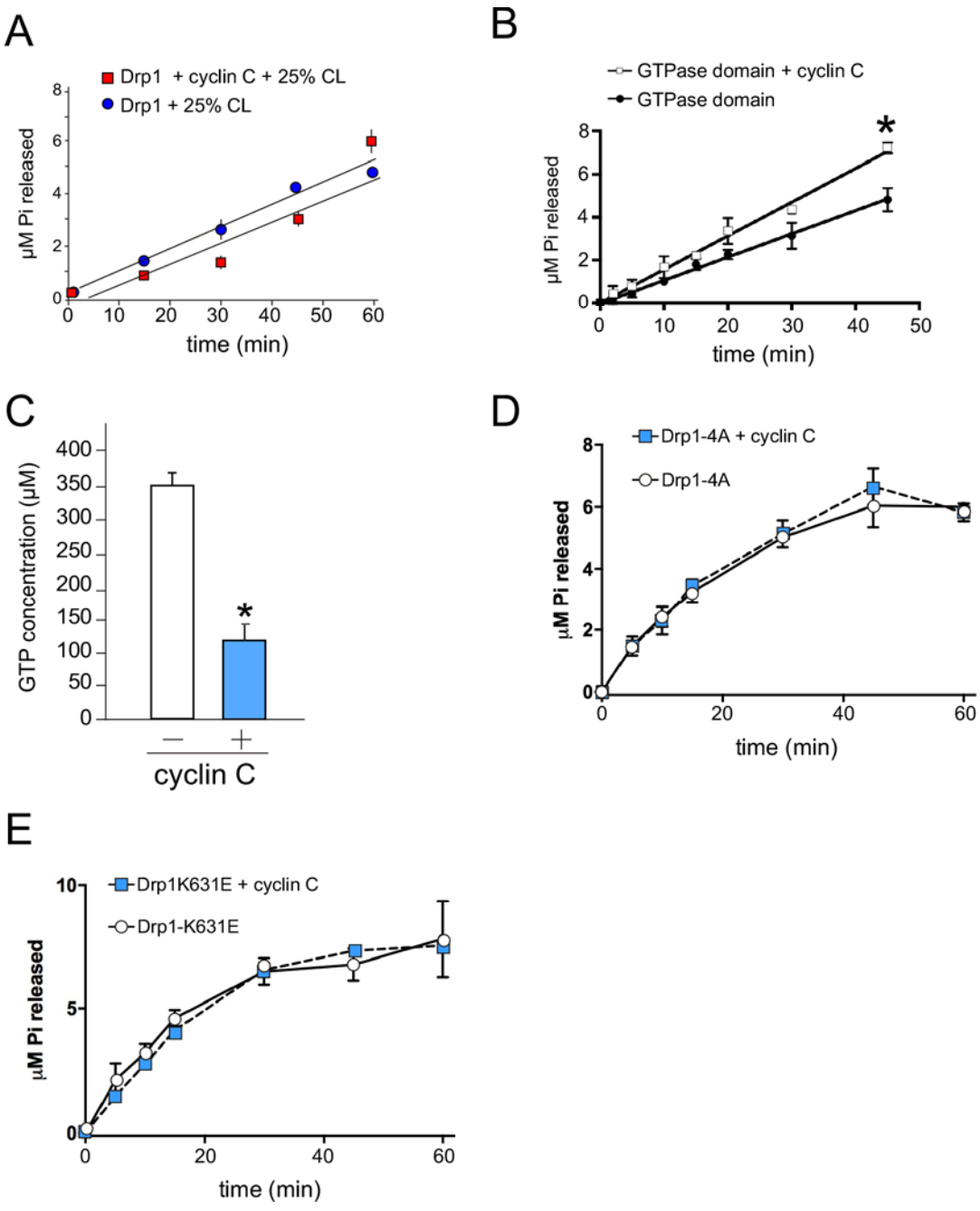

Figure S4. Cyclin C stimulates GTPase domain activity but not filament defective mutants. (A) GTPase activity was measured with 1 µM Drp1 + 25% CL with and without 5 µM cyclin C as indicated. (B) The experiments in (A) were repeated with the GTPase domain of Drp1 with 0.5 mM GTP. Asterisk indicates $p<0.01$. (C) Km calculation for GTP binding of the

GTPase domain with and without cyclin C. Asterisk indicates $p<0.02$. (D, E) GTPase assays were conducted as before with the dimer only (Drp1-4A) and monomer only (K631E) derivatives with and without cyclin C as indicated.