Figure S1. Relative distribution of NDE1/NDEL1 and CENP-F during late G2 and mitosis. HeLa cells were stained for endogenous NDE1/NDEL1 and CENP-F to determine their localization pattern. Endogenous NDE1/NDEL1 and CENP-F colocalized at the nuclear NE in G2 and at the kinetochore from prophase until mid-anaphase. Bars, 5 µm.
Figure S2. **Effect of in Nde1 mutants on chromosome behavior. (A–F)** Representative images from the live analysis of Fig. 4 (A–F) showing H2B-RFP HeLa cells expressing GFP, GFP WT Nde1, GFP Nde1 phosphomimetic, GFP Nde1 phosphomutant, GFP Nde1-del-dynein, or GFP Nde1-del-Lis1. Image panels represent time points that are 9 min apart. Bars, 10 µm.
Figure S3. Effect of Ndel1 RNAi rescue on chromosome behavior. (A–G) Representative images from the live analysis of Fig. 4 (H–N) showing H2B-RFP HeLa cells treated for 48 h with either control siRNA or Nde1 siRNA and then transfected with GFP, GFP WT Nde1, GFP Nde1-3E, GFP Nde1-3A, GFP Nde1-del-dynein, or GFP Nde1-del-Lis1. Bars, 10 µm. (H) Western blot analysis of HeLa cell lysate treated for 48 h with control siRNA, Nde1 siRNA, or Nde1 siRNA rescued with GFP WT Nde1, GFP Nde1-3E, or GFP Nde1-3A confirms the successful reduction in Nde1 protein levels.