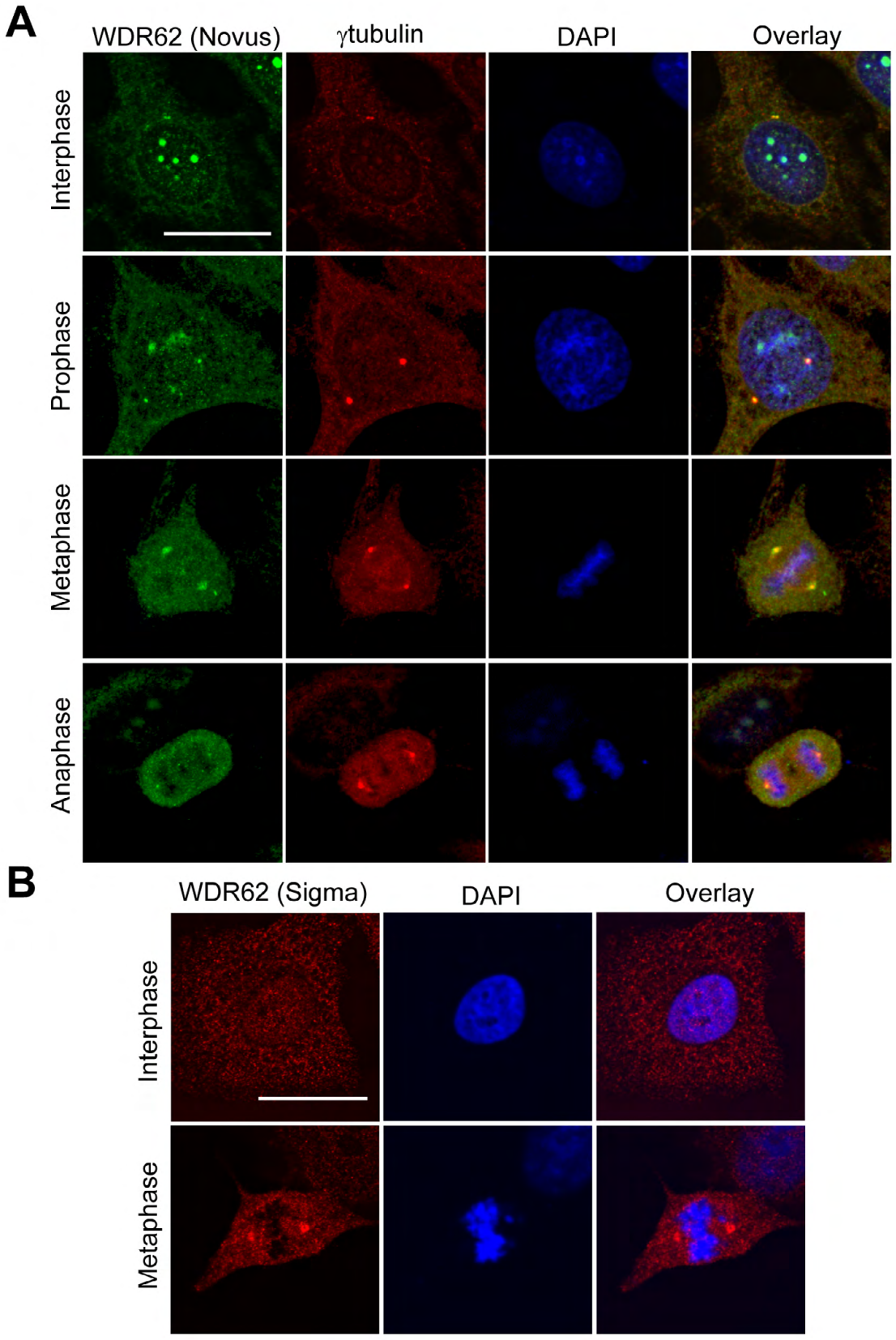

Fig. S1. WDR62 recruitment to the mitotic spindle. (A) WDR62 subcellular localization in HeLa cells at different stages of mitosis was determined by immunofluorescent staining with a polyclonal antibody raised against WDR62 aa750-800 (Novus Biologicals #NB100-77302). Condensed chromatin was stained with DAPI and γ-tubulin immunostaining revealed centrosomes and spindle poles. (B) HeLa cells at interphase or metaphase were stained with a monoclonal antibody (Sigma, W3269) raised against aa1-208 of WDR62. Scale bars: 20 μm.

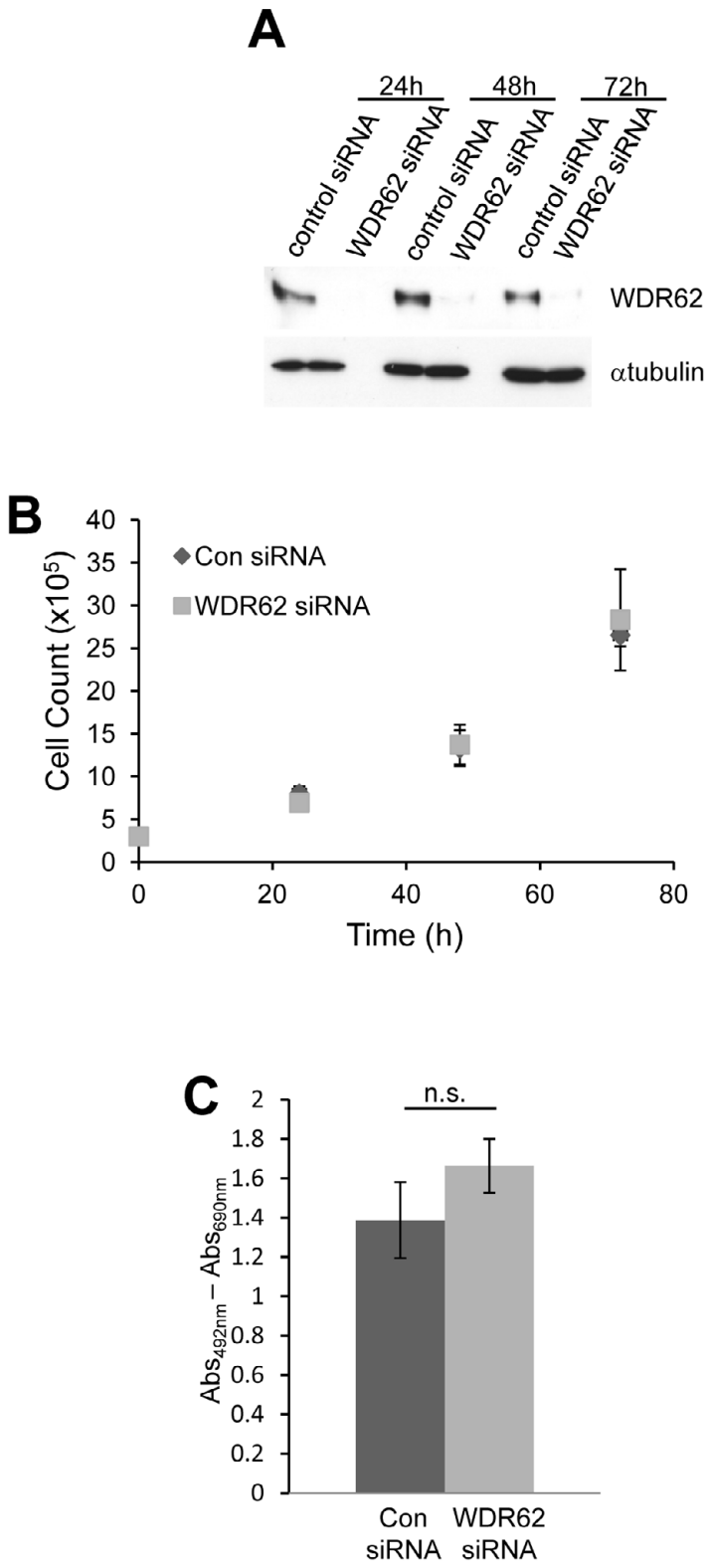

Fig. S2. WDR62 knockdown effect on cell division. (A) HeLa cells were transfected with WDR62 siRNA, lysed at 24, 48 or 72 h post-transfection and immunoblotted for WDR62 and α-tubulin. (B) The proliferation of HeLa cells transfected with WDR62 siRNA or a non-targeting control siRNA (Con siRNA) was measured by cell counting at 24, 48 and 72 h post-transfection. Values are mean ± s.d. from 3 independent experiments. (C) The viability of HeLa cells transfected with Con siRNA or WDR62 siRNA was measured by XTT assay at 48 h post-transfection. Absorbance values were mean ± s.d. from three independent experiments.

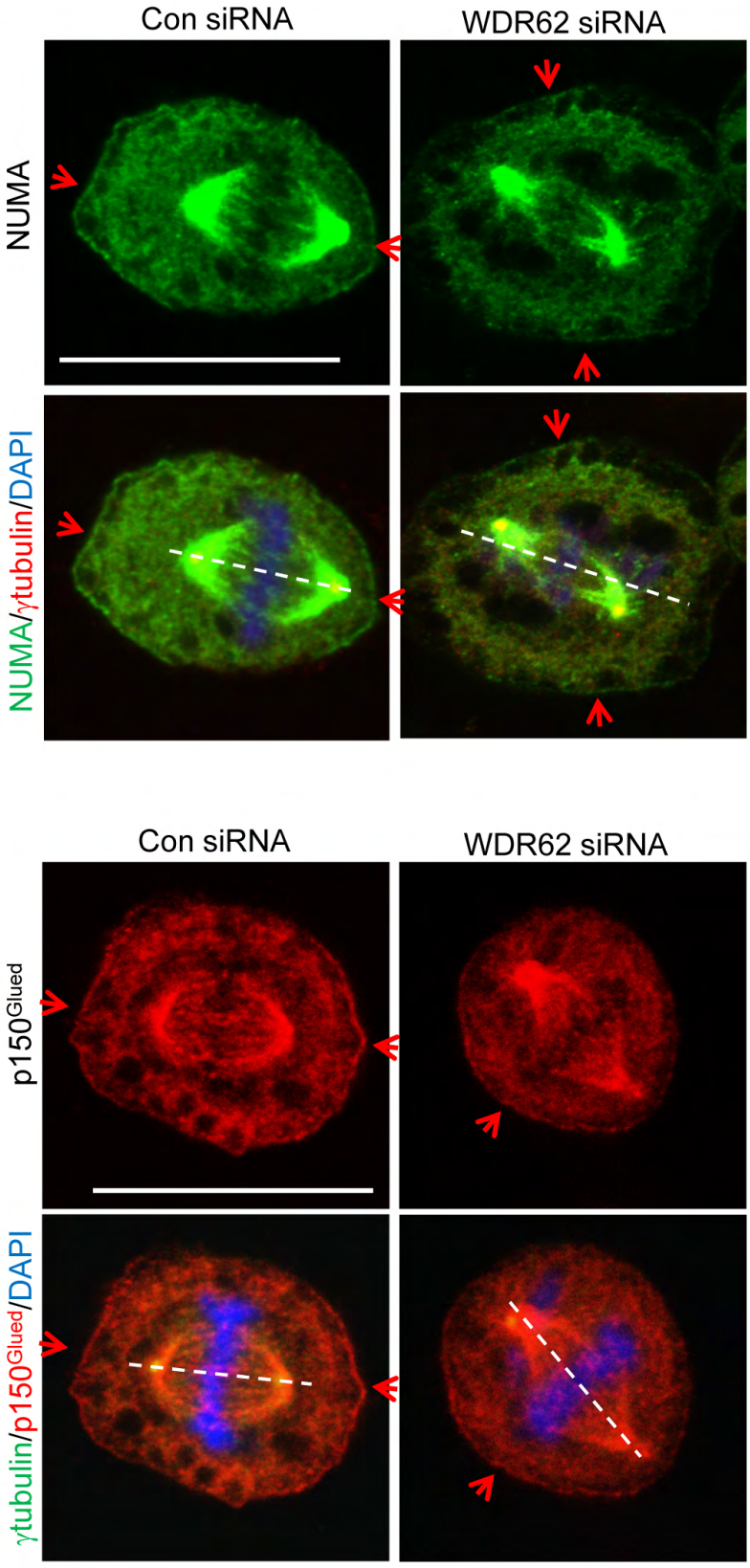

Fig. S3. Mitotic localization of NUMA and p150^{Glued} is unaffected by WDR62 loss. HeLa cells were treated with WDR62 siRNA or a control siRNA and fixed at 8 h following release from DTB. Cells were stained for NUMA or p150^{Glued}. Cells were co-stained with γ-tubulin and DAPI. Scale bars: 20 μm. Red arrows indicate cortical localization of NUMA or p150^{Glued}. Broken lines indicate spindle polarity axis.

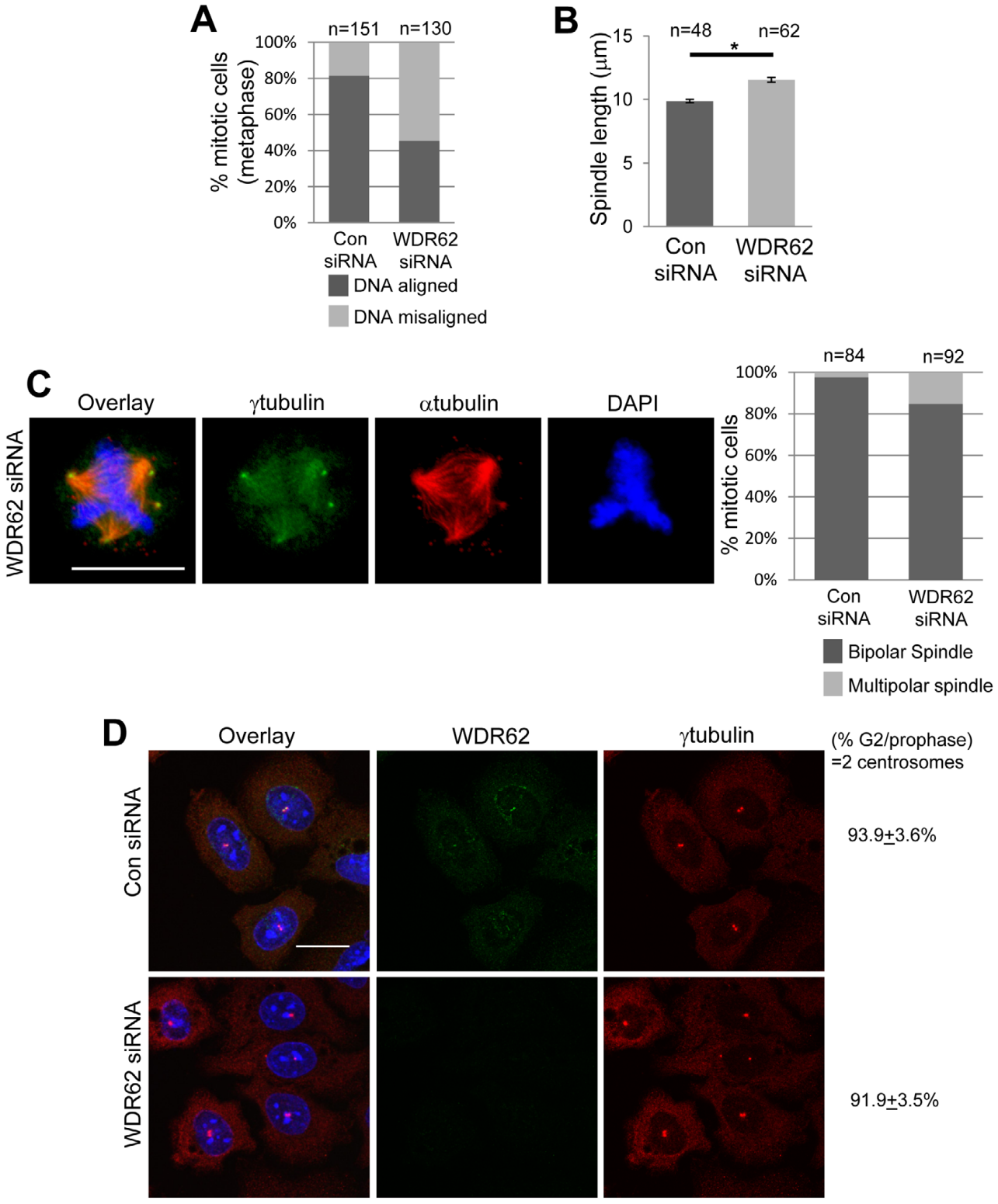

Fig. S4. WDR62 depletion effects on chromosome congression, spindle length and multipolar spindle formation. (A) The proportion of metaphase HeLa cells with misaligned DNA was counted in Con siRNA and WDR62 siRNA-treated cells. (B) Metaphase spindle length was determined by α-tubulin staining and measuring from pole-to-pole. Graph indicates mean ± s.d., 'P<0.0001. All 'n' values represent total cells counted from three independent experiments. (C) A representative image of multipolar spindle in WDR62-depleted HeLa cell visualized by α- and γ-tubulin co-staining. Proportion of cells with multi-polar spindles in synchronized (DTB) control or WDR62-depleted HeLa cells were measured. 'n' values are total cells counted from three independent experiments. (D) Thymidine synchronized HeLa cells, treated with WDR62 siRNA or Con siRNA, were fixed prior to mitosis (7 h post thymidine release) and stained for WDR62 and γ-tubulin. Cells with two centrosomes or >2 centrosomes were manually scored. The proportion of total cells with 2 centrosomes was expressed as mean ± s.d. from three independent experiments with at least 100 cells scored per experiment.

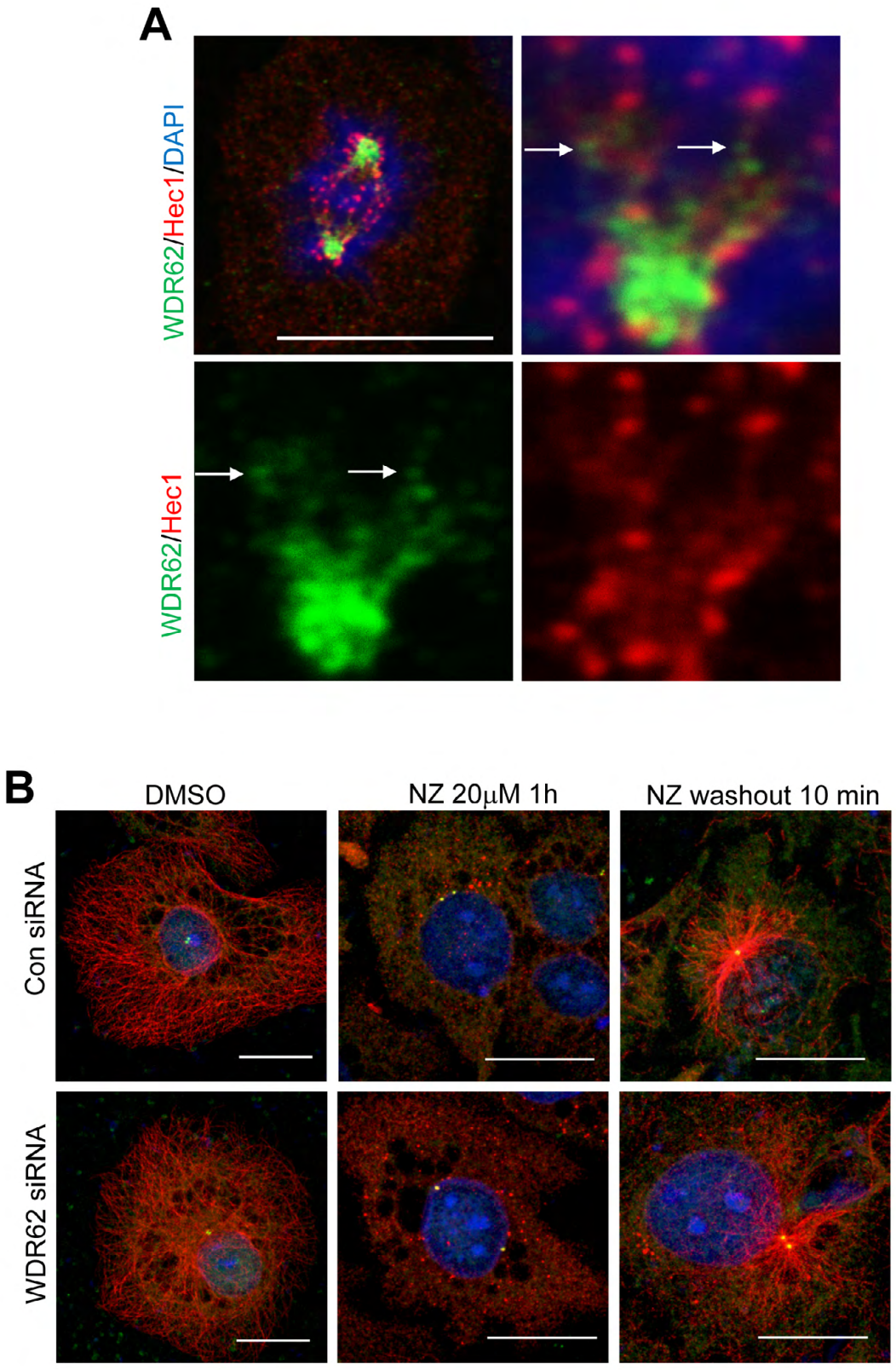

Fig. S5. WDR62 decorates kinetochore microtubules and is not required for microtubule formation in interphase. (A) Synchronized HeLa cells (DTB) were $CaCl_2$ extracted to reveal kinetochore microtubules. WDR62 localization was determined by immunofluorescence while kinetochores were marked by staining for centromeric protein Hec1. Arrows highlight WDR62 puncta present on kinetochore microtubules. (B) Interphase microtubules of WDR62 siRNA and Con siRNA-treated HeLa cells were depolymerized with nocodazole (20 μM, 1 h). Microtubule regrowth was by nocodazole washout for 10 min. Cells were fixed and stained for α- and γ-tubulin. Scale bars: 20 μm.

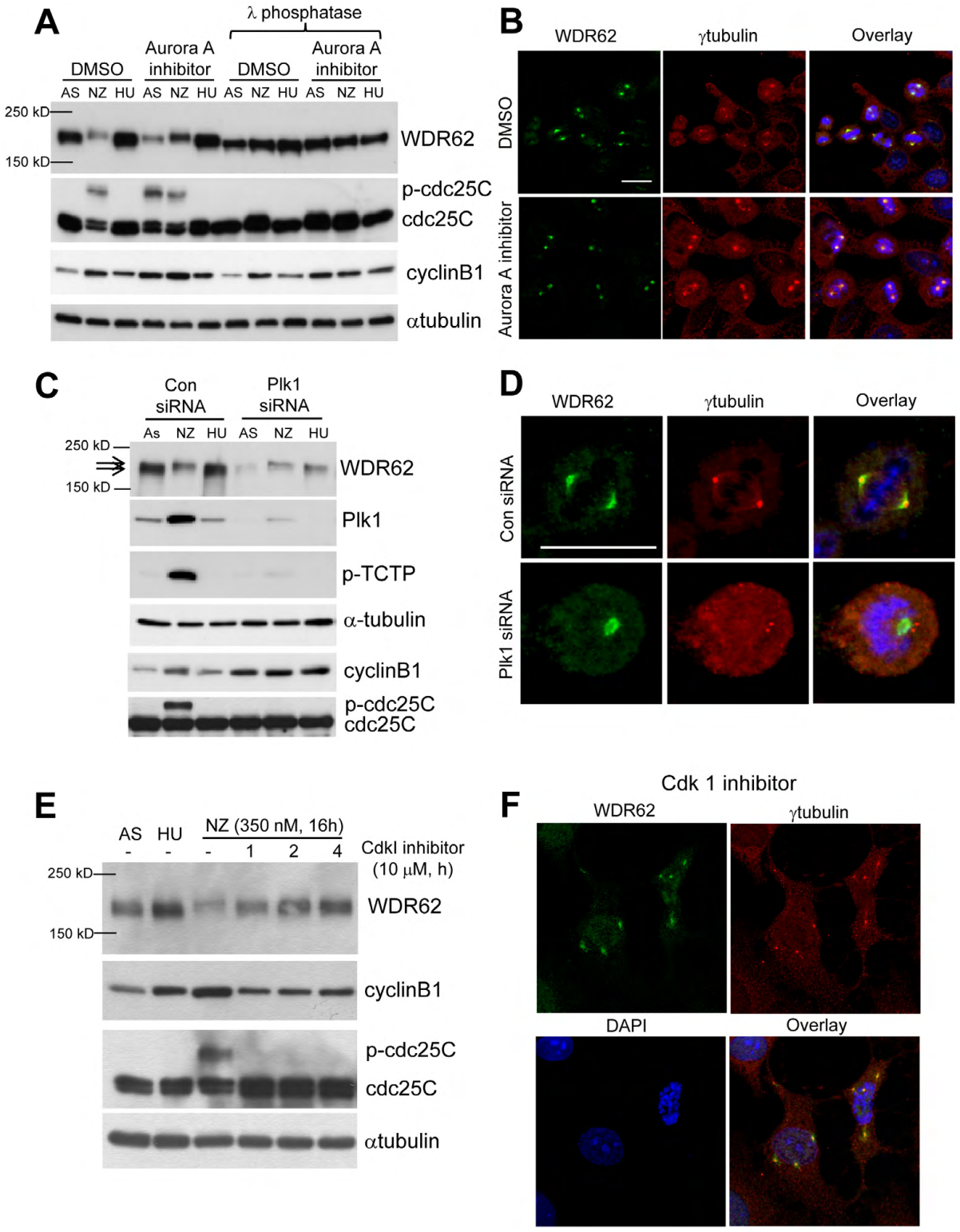

Fig. S6. WDR62 phosphorylation during prometaphase arrest. (A) HeLa cells were pre-treated with Aurora A inhibitor (2 μM) before additional treatment with nocodazole (NZ, 350 nM, 16 h) or hydroxyurea (HU, 2 mM, 16 h) or left unsynchronized (AS) and immunoblotted as indicated. Cell lysates were also λ-phosphatase (400 U, 20 min, 30°C)-treated to confirm WDR62 phosphorylation. (B) Synchronized HeLa cells (DTB) were released into the presence of Aurora A inhibitor (2 μM) or a vehicle control (DMSO, 0.1% v/v). Cells were fixed at 8 h following thymidine release and stained for WDR62 and γ-tubulin. Scale bars: 20 μm. (C) HeLa cells were transfected with Plk1 siRNA or non-targeting siRNA (Con siRNA) and synchronized with nocodazole (350 nM, 16 h), hydroxyurea (2 mM, 16 h) or left unsynchronized (AS) before immunoblot analysis. (D) Plk1 siRNA- or Con siRNA-transfected HeLa cells were fixed and stained for WDR62 and γ-tubulin. (E) M-phase arrested HeLa cells (NZ, 350 nM, 16 h) were treated with Cdk1 Inhibitor I (10 μM) for indicated times and samples immunoblotted for WDR62, α-tubulin, cyclin B1 and cdc25C. (F) Thymidine synchronized HeLa cells were released into the presence of a Cdk1 inhibitor I (10 μM) or a vehicle control (DMSO, 0.1% v/v). Cells were fixed at 8 h following thymidine release and stained for WDR62 and γ-tubulin.

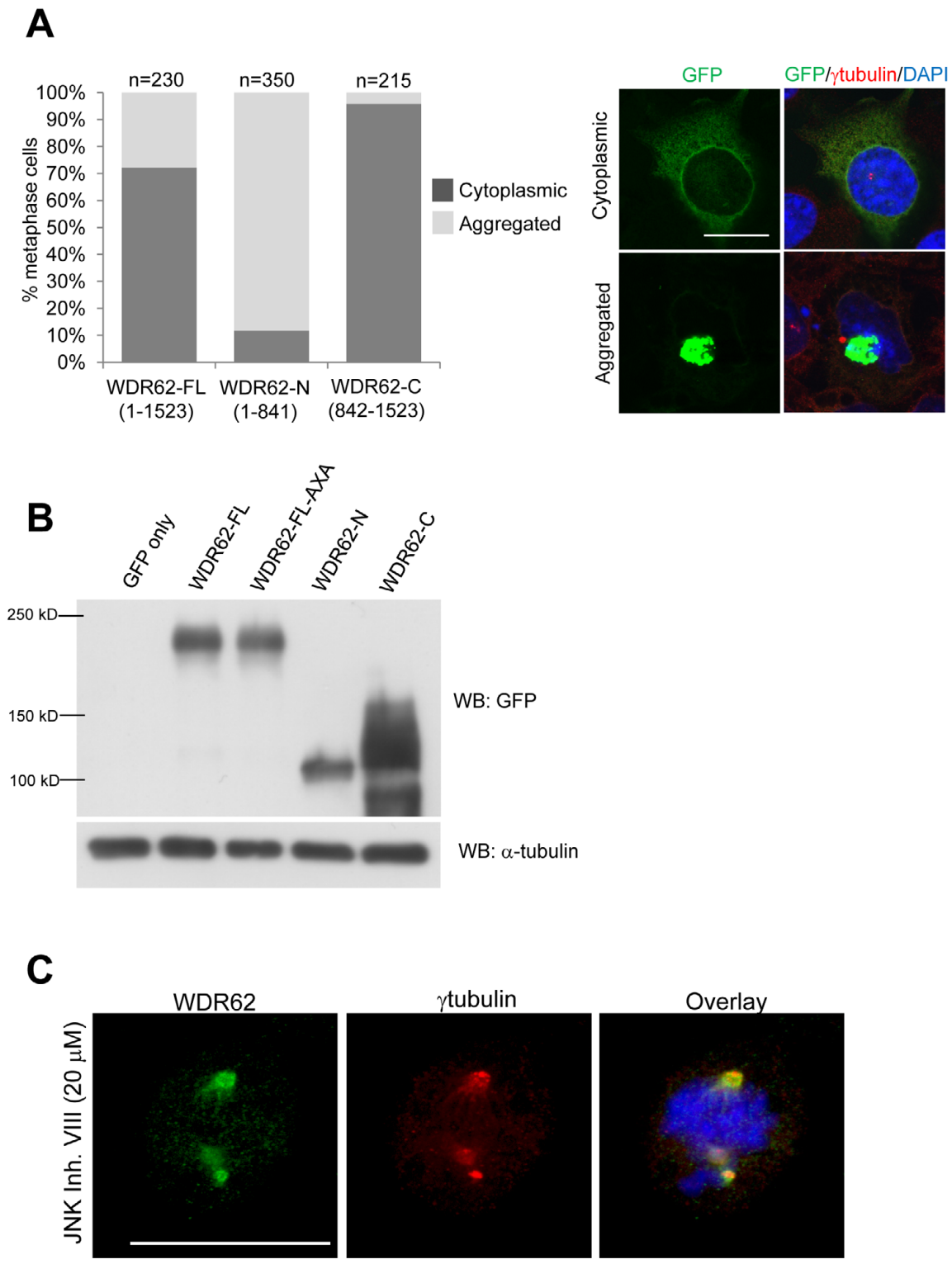

Fig. S7. Expression and cytoplasmic distribution of GFP-labeled WDR62 in Ad293 cells. (A) Ad293 cells were transiently transfected with GFP-labelled full length WDR62 (WDR62-FL, aa1-1523) and truncation mutants containing N- (WDR62-N, aa1-841) and C-terminal (WDR62-C, aa842-1523) regions of WDR62. The proportion of interphase cells with cytoplasmic or aggregated GFP distribution was determined. Representative images of cytoplasmic or aggregated GFP distribution are shown. 'n' values represent total cells counted from two independent experiments. (B) Total protein expression of WDR62-FL, WDR62-FL-AXA and truncation mutant (WDR62-N, WDR62-C) was also determined by immunoblot analysis with α-GFP. (C) HeLa cells were synchronized (DTB) and released into media containing JNK inhibitor VIII (20 μM). Mitotic cells were fixed and stained for WDR62 and γ-tubulin.