Expanded View Figures

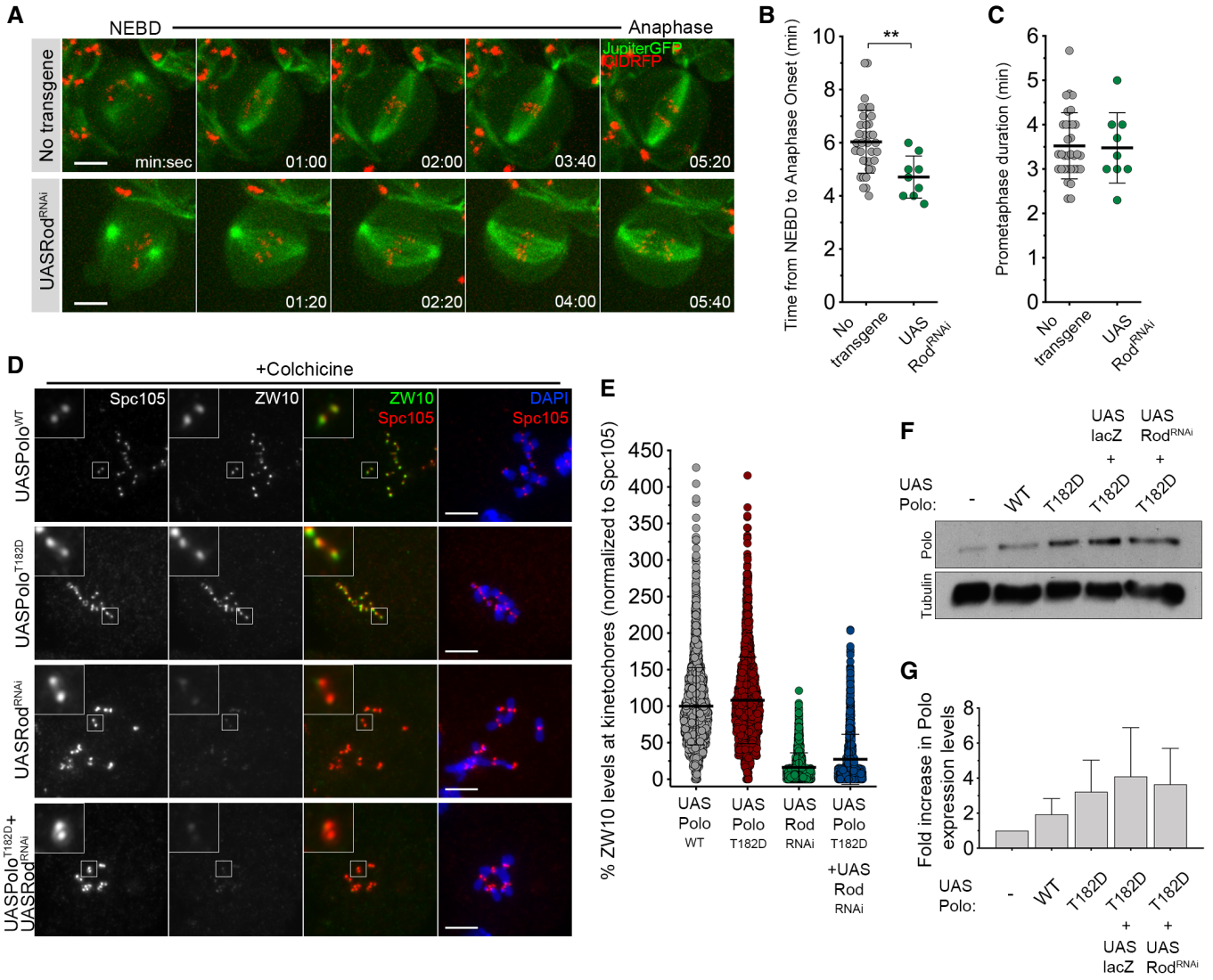


Figure EV1. Absence of the RZZ complex does not impact on the time required for chromosome congression in *Drosophila* neuroblasts.

A Selected stills from live imaging analysis of mitotic progression in neuroblasts depleted of Rod (UASRod^{RNAi}). Neuroblasts without transgene expression were used as control.

B Quantification of the mitotic time (time from NEBD to anaphase onset) for neuroblasts shown in (A) ($n \geq 9$ neuroblasts for each condition, $n = 2$ independent experiments).

C Quantification of the time spent in prometaphase (from NEBD until last KT alignment at the metaphase plate) for neuroblasts shown in (A) ($n \geq 9$ neuroblasts for each condition, $n = 2$ independent experiments).

D Representative immunofluorescence images of ZW10 levels in colchicine-treated neuroblasts expressing UASPolo^{WT}, UASPolo^{T182D}, UASRod^{RNAi} or UASPolo^{T182D} in a Rod-depleted background (UASPolo^{T182D} + UASRod^{RNAi}). Insets show magnifications of the outlined regions. Neuroblasts expressing UASPolo^{WT} were used as control.

E Graph represents ZW10 levels in neuroblasts for the conditions shown in (D). ZW10 levels were determined relative to Spc105, and all values were normalized to the control mean fluorescence intensity, which are set to 100% ($n \geq 1116$ KTs for each condition, $n \geq 2$ independent experiments).

F Western blot analysis of UAS dilution effect on Polo overexpression levels in brain extracts from 3rd-instar larvae expressing UASPolo^{WT}, UASPolo^{T182D}, UASPolo^{T182D} together with UASlacZ or in a Rod-depleted background (UASPolo^{T182D} + UASRod^{RNAi}). Tubulin was used as a loading control.

G Graph represents the fold increase in Polo protein levels relative to the absence of transgene overexpression. Expected Polo protein levels were determined after normalization to tubulin levels, and fold increase was calculated comparing to the actual Polo levels.

Data information: Statistical analysis was calculated using an unpaired *t*-test (Mann–Whitney). *P* values: **< 0.01. Data are shown as mean ± SD. Scale bar: 5 μm.

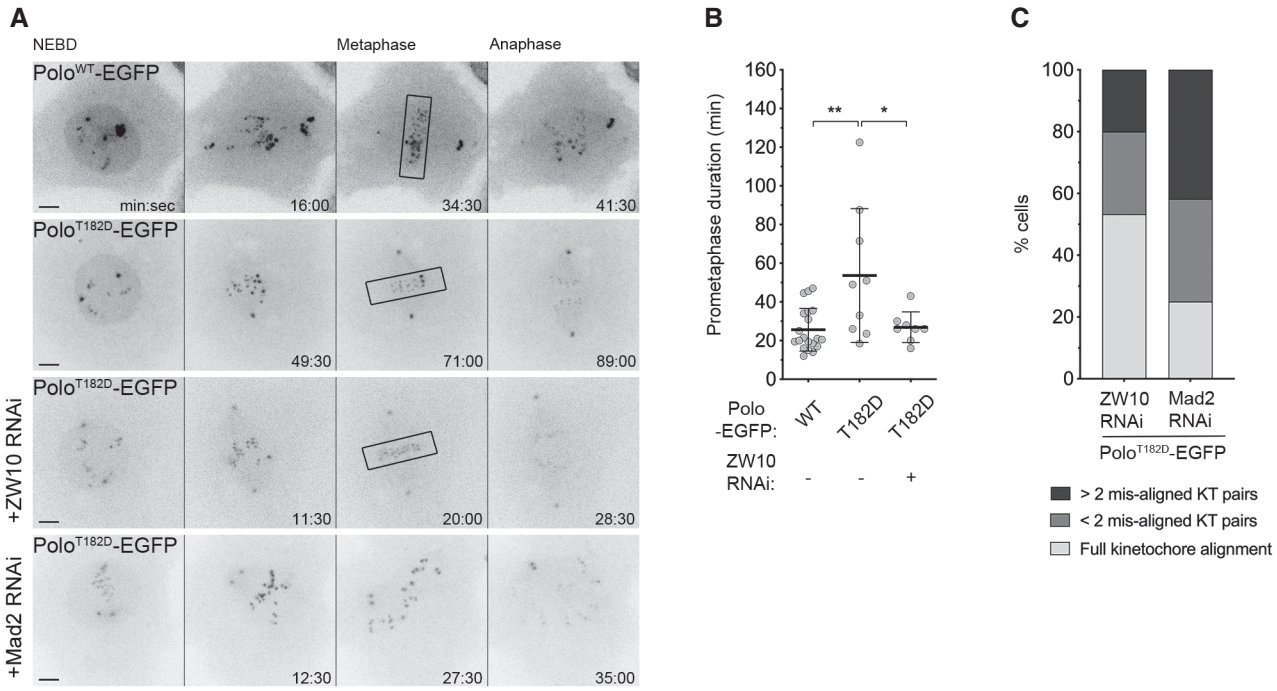


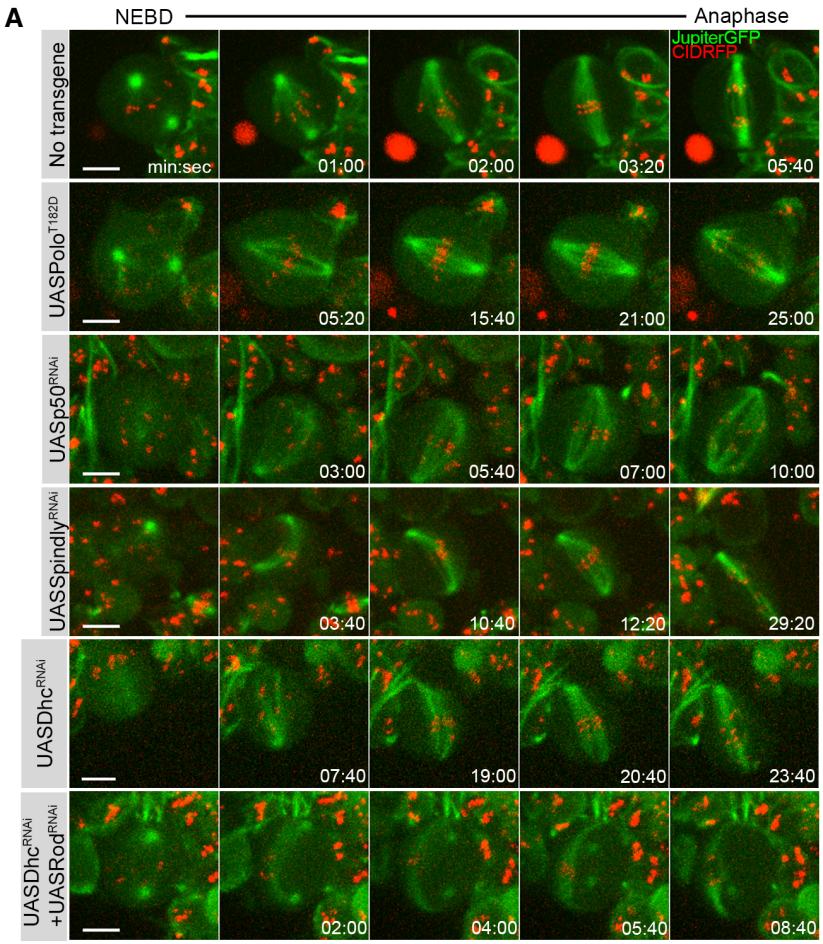
Figure EV2. Depletion of ZW10 rescues chromosome congression in Polo^{T182D} expressing cells independently of SAC impairment.

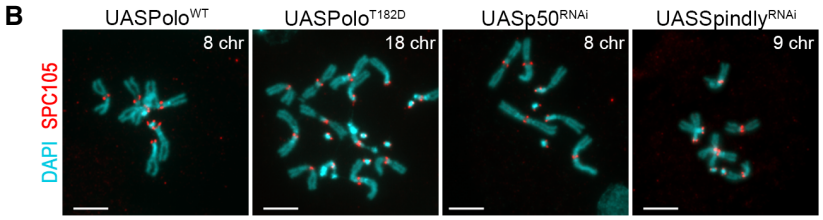
A Selected stills from live imaging analysis of mitotic progression in *Drosophila* S2 cells expressing Polo^{WT}-EGFP, Polo^{T182D}-EGFP and Polo^{T182D}-EGFP in a ZW10- or Mad2-depleted background. Time 0 refers to nuclear envelope breakdown (NEBD). Black box highlights full KT alignment.

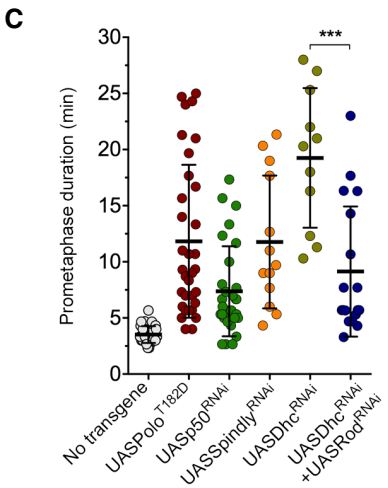
B Quantification of the time spent in prometaphase (from NEBD until last KT alignment at the metaphase plate) for cells shown in A ($n \geq 8$ cells for each condition, $n \geq 3$ independent experiments). Note that the Mad2-depleted cell does not achieve full KT alignment.

C Graph represents the percentage of cells that enter anaphase with different levels of KT alignment ($n = 15$ cells, $n \geq 3$ independent experiments).

Data information: Statistical analysis was calculated using a Kruskal–Wallis test for multiple comparisons. *P* values: *< 0.05; **< 0.01. Data are shown as mean ± SD. Scale bar: 5 μm.







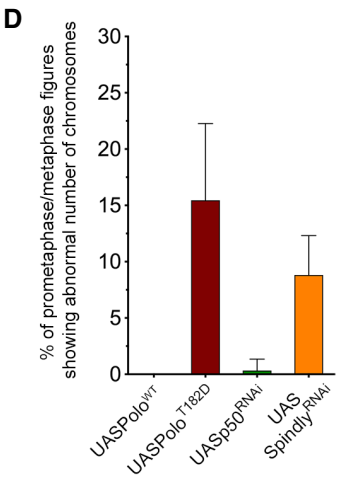


Figure EV3. Spindly depletion and expression of Polo^{T182D} result in similar mitotic phenotypes.

A Selected stills from live imaging analysis of mitotic progression in neuroblasts expressing UASPolo^{T182D}, or depleted of p50 (UASp50^{RNAi}), Spindly (UASSpindly^{RNAi}), dynein heavy chain (UASDhc^{RNAi}) or Rod in Dhc-depleted background (UASDhc^{RNAi} + UASRod^{RNAi}).

B Representative immunofluorescence images of mitotic neuroblasts from squashed brains showing distinct chromosome content. Spc105 was used as a KT reference. Neuroblasts were identified as the larger cells. Chromosome content is shown for each neuroblast.

C Quantification of the time spent in prometaphase (from NEBD until last KT alignment at the metaphase plate) for neuroblasts shown in (A) ($n \geq 11$ neuroblasts for each condition, $n \geq 6$ independent experiments).

D Graph represents the % of aneuploidy among mitotic figures for conditions shown in (C). For the quantification of aneuploidy, the whole brain was analysed and prometaphase and metaphase mitotic neuroblasts were scored for chromosome number (aneuploid cell when > 8 chromosomes (chr) were visualized). Polo^{WT} neuroblasts were used as control ($n \geq 305$ neuroblasts from at least six larvae brains for each condition, $n \geq 3$ independent experiments).

Data information: Statistical analysis was calculated using an unpaired *t*-test (Mann–Whitney). *P* values: ***< 0.001. Data are shown as mean ± SD. Scale bar: 5 μm.

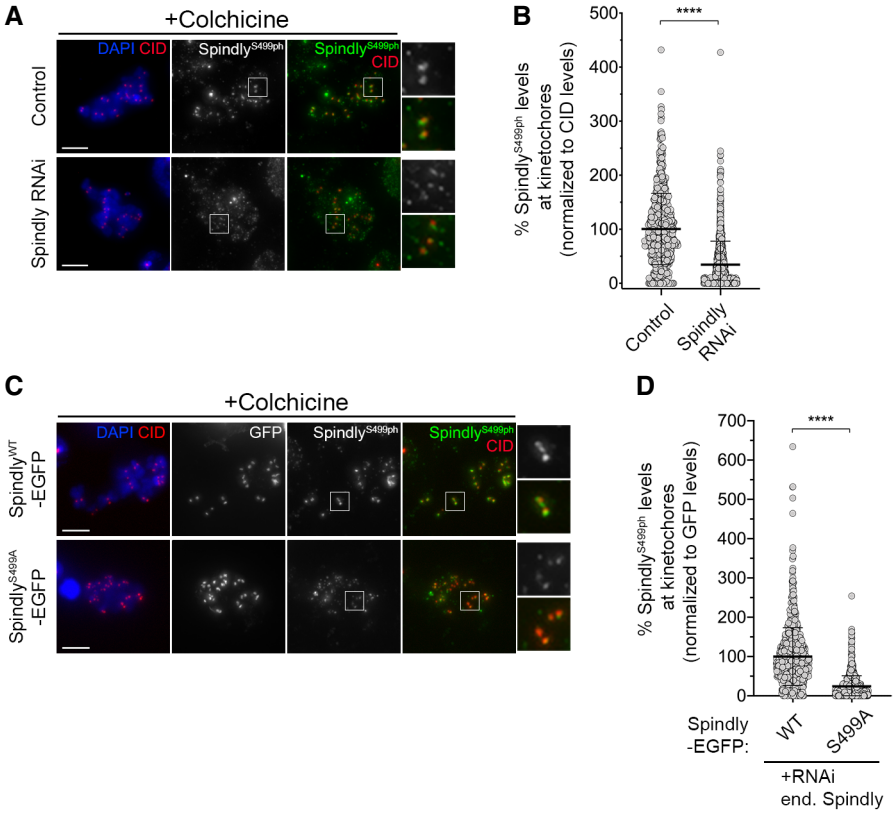


Figure EV4. Phospho(ph)Ser499 antibody recognizes a specific epitope on Spindly.

A Representative immunofluorescence images of Spindly-phospho(ph)Ser499 levels at unattached KTs in control *Drosophila* S2 cells and in Spindly-depleted cells. Insets display magnifications of the outlined regions. Cells were treated with colchicine prior to fixation to generate unattached KTs. CID was used as a KT reference.

B Graph represents the levels of Spindly phS499 at unattached KTs for cells shown in (A). Spindly phS499 levels were determined relative to CID, and all values were normalized to the control mean fluorescence intensity, which was set to 100% ($n \geq 701$ KTs from at least 35 cells for each condition, $n = 2$ independent experiments).

C Representative immunofluorescence images of Spindly phS499 levels at unattached KTs in *Drosophila* S2 cells expressing either Spindly^{WT}–EGFP or Spindly^{S499A}–EGFP and depleted of endogenous Spindly. Insets display magnifications of the outlined regions. Cells were treated with colchicine prior to fixation to generate unattached KTs. CID was used as a KT reference.

D Graph represents the levels of Spindly phS499 at unattached KTs for cells shown in (C). Spindly phS499 levels were determined relative to GFP, and all values were normalized to the mean fluorescence intensity levels quantified in Spindly^{WT}–EGFP expressing cells, which was set to 100% ($n \geq 824$ KTs from at least 42 cells for each condition, $n = 2$ independent experiments).

Data information: Statistical analysis was calculated using an unpaired *t*-test (Mann–Whitney). *P* values: ****< 0.0001. Data are shown as mean ± SD. Scale bar: 5 μm.