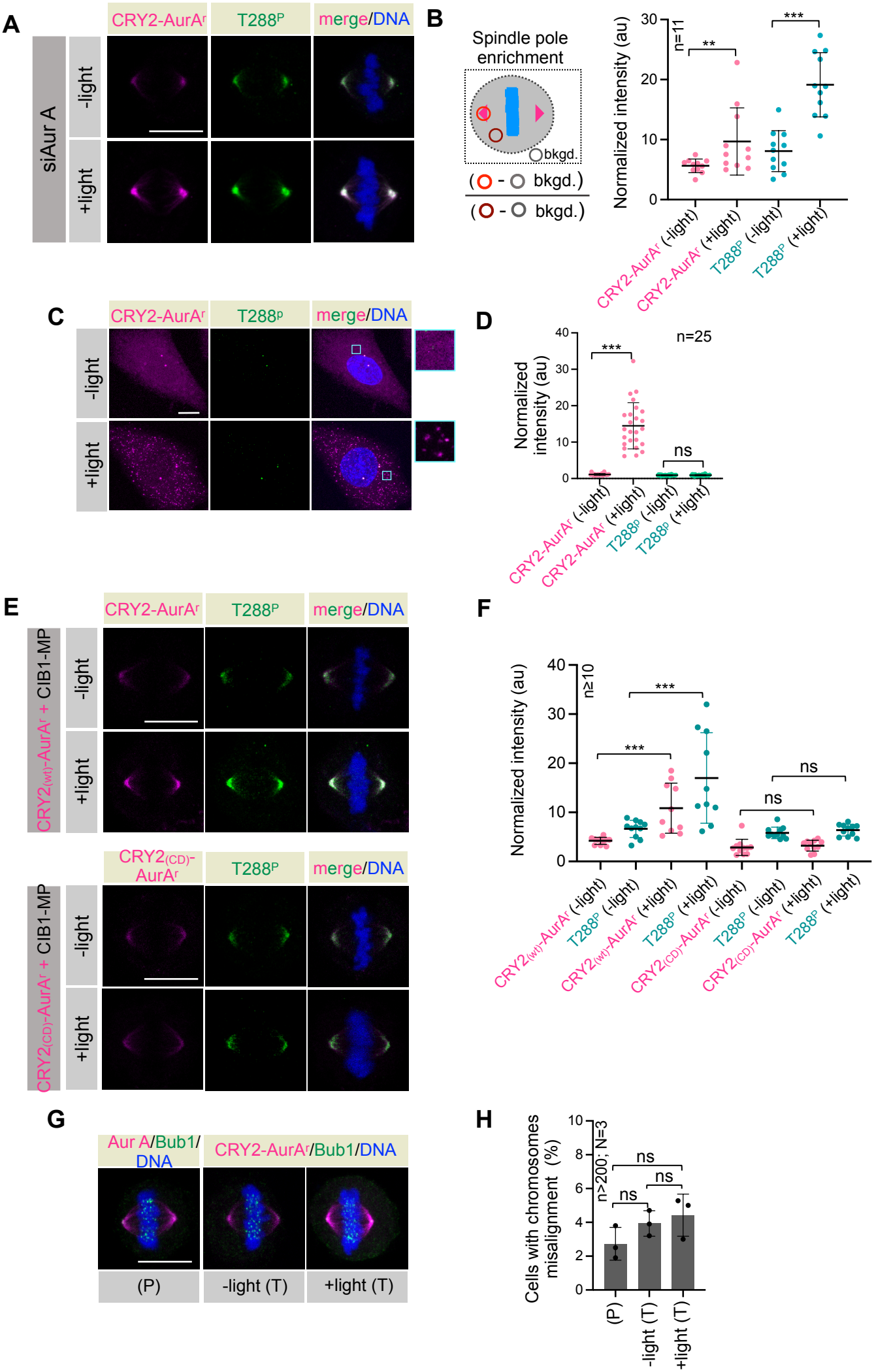
Appendix data for:

Light-induced spindle assembly (LISA) through Aurora A clustering

Table of contents **Page**

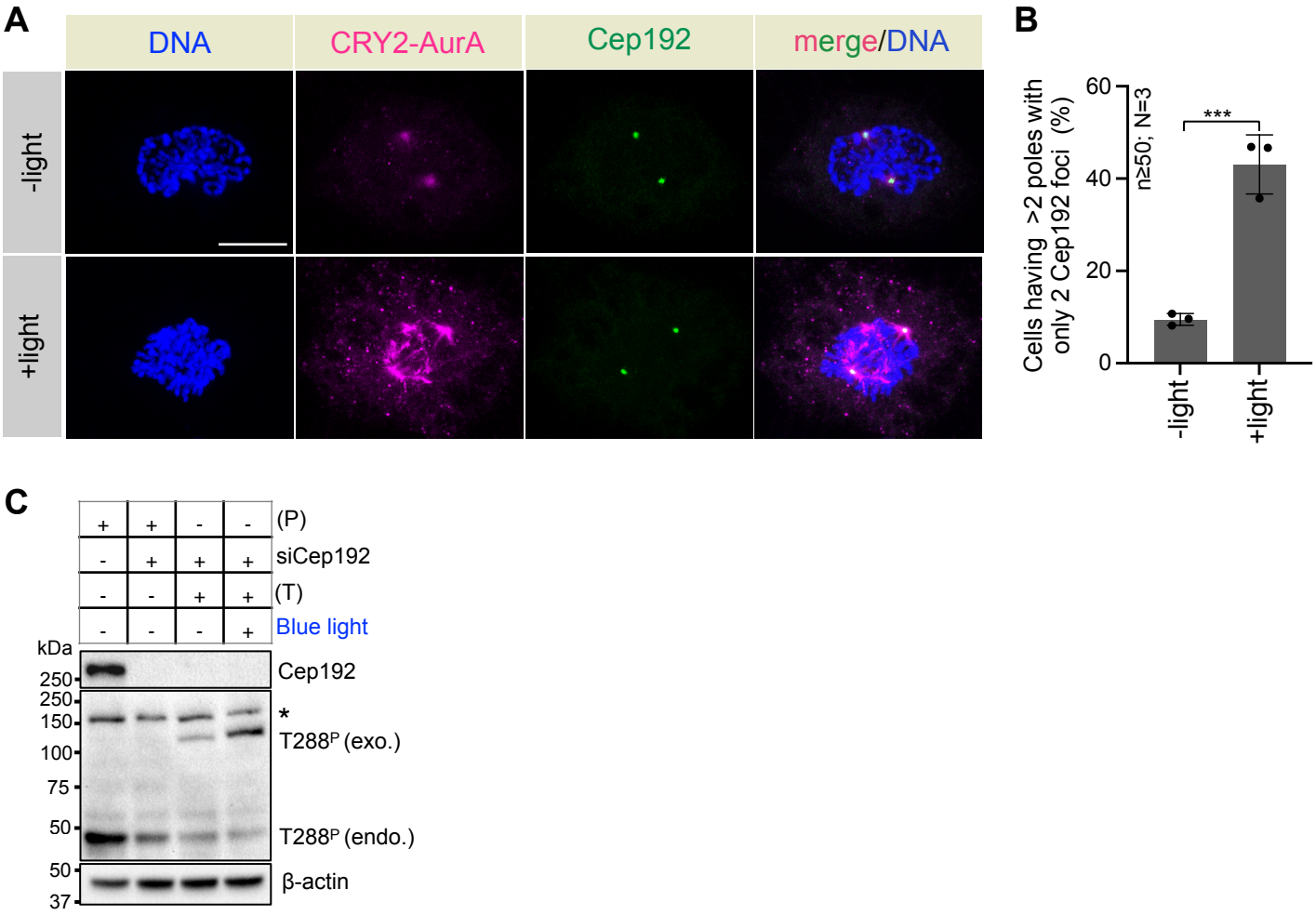
Appendix Fig. S1



Appendix Figure S1

Clustering of Aurora A is critical for its autophosphorylation at the T-loop in mitosis

(A, B) Immunofluorescence (IF) microscopy analysis of HeLa cells stably coexpressing CRY2-AurA^{r} and CIB1-MP were depleted for Aurora A for 60 h. These cells were then left either unexposed or exposed to blue light, as indicated. Cells were stained with anti-mCherry (magenta) and anti-T288^{P} (green) antibodies. DNA is shown in blue (A). Schematic showing the method of quantification of spindle pole intensity in metaphase cells, and the outcome of such analysis on the right (B). Error bars: mean ± SD (n=11). Exact p values from left to right are **p=0.0092, ***p<0.001.
(C, D) IF analysis of HeLa cells stably coexpressing CRY2-AurA^{r} and CIB1-MP in interphase in the absence and presence of blue light, as indicated. These cells were stained with anti-mCherry (magenta) and anti-T288^{P} (green) antibodies. DNA is shown in blue. Rectangular regions marked in the image are shown magnified on the top right (C). Quantification of CRY2-AurA^{r} and T288^{P} intensity (in au) within clustered CRY2-AurA^{r} in the absence or presence of blue light (D). Error bars: mean ± SD (n=25). Exact p values from left to right are ***p<0.001, and ns-p=0.6767.
(E, F) IF analysis of HeLa cells in metaphase transiently transfected with CRY2-AurA^{r} or CRY2$_{(CD)}$-AurA^{r} together with CIB1-MP, before and after blue light illumination, stained with anti-mCherry (magenta) and T288^{P} (green) antibodies. DNA is shown in blue (E). Quantification of spindle pole intensity as shown for panel S2B in metaphase cells (F). Error bars: mean ± SD (n≥10). Exact p values from left to right are ***p<0.001, ***p<0.001, p=0.5564 (ns), p=0.2794 (ns).
(G, H) IF analysis of parental (P) or transgenic (T) HeLa, either without or on exposure with blue light, as indicated, were stained with either anti-AurA (magenta) or anti-mCherry (magenta) together with anti-Bub1 (green) to assess chromosome misalignment defects (G), the quantification of this assessment is shown on the right (H). Exact p values from left to right are p=0.1657 (ns), p=0.1366 (ns), p=0.5879 (ns).
p value is denoted as follows: ns-p≥0.05; **p<0.01; ***p<0.001 as determined by two-tailed unpaired Student's t-test. Scale bars in (A, C, E, G) represent 10 μm.

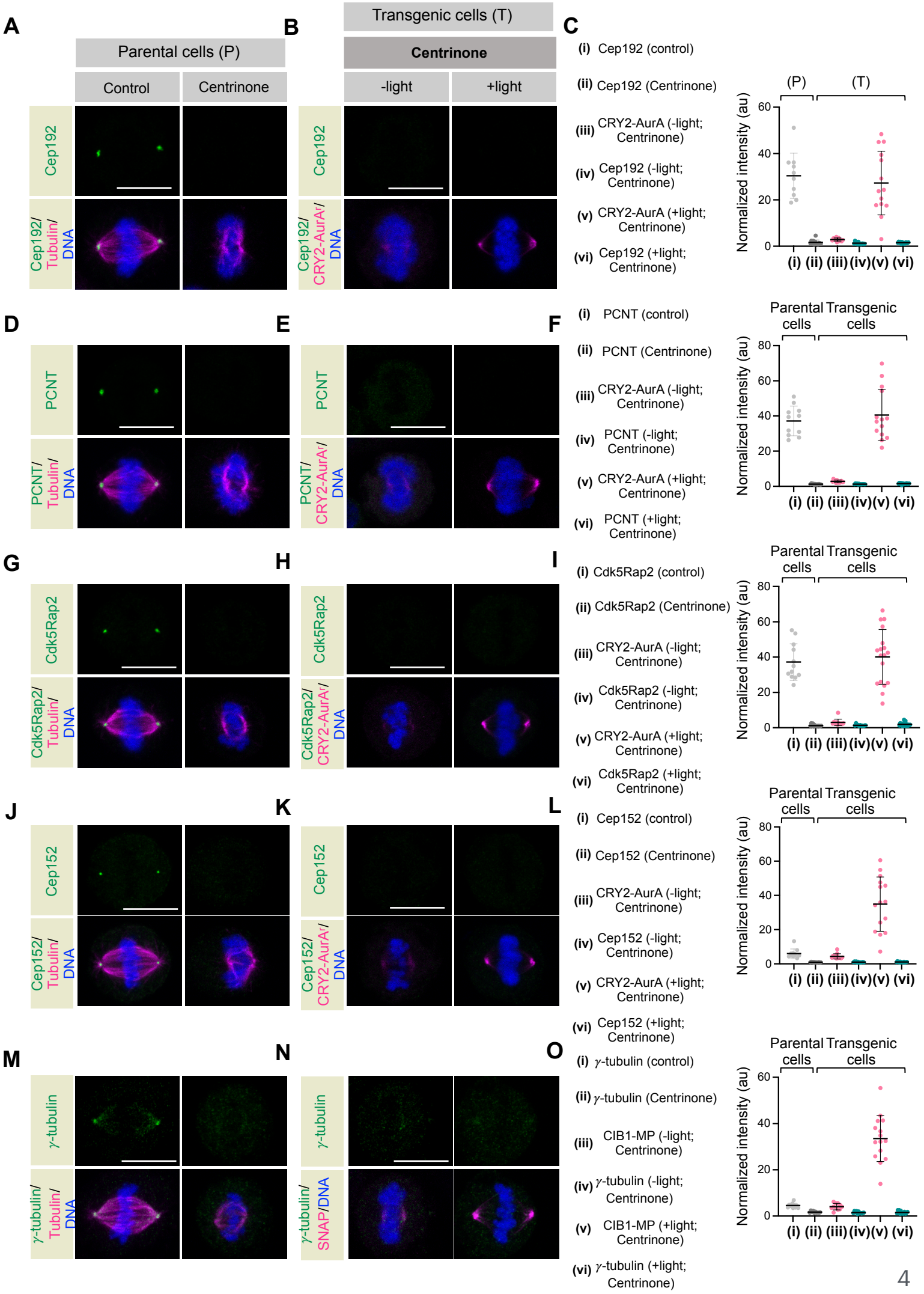


Appendix Figure S2

<u>Cep192 does not localize with ectopic CRY2-AurA^r-based clusters</u>

(A, B) IF analysis of HeLa cells stably coexpressing CRY2-AurA^r and CIB1-MP synchronized in prophase in the absence and presence of blue light, as indicated (see Fig. 3A for the method of synchronization). These cells were stained with anti-mCherry (magenta) and anti-Cep192 (green) antibodies. DNA is shown in blue (A). Note that Cep192 staining is restricted to the centrosomes, and do not colocalize with CRY2-AurA^r-based clusters in cells briefly exposed to blue light. The quantification is shown towards the right (B). Exact p value is ***p<0.001.
(C) Immunoblot analysis of protein extracts prepared from mitotically synchronized control (parental cells-P) and cells stably coexpressing CRY2-AurA^r and CIB1-MP (transgenic cells-T), in the presence or absence of Cep192 siRNA, and in the absence or upon exposure to blue light, as indicated. Extracts were probed with antibodies directed against Cep192, T288^P, and β-actin. Endogenous and exogenous T288^P bands are indicated. Asterisks indicate non-specific bands. Note the increase in T288^P (exo.) band intensity upon blue light in cells depleted for Cep192, compared to no light illumination.
p value is denoted as ***p<0.001 as determined by two-tailed unpaired Student's t-test. Scale bars in (A) represent 10 μm

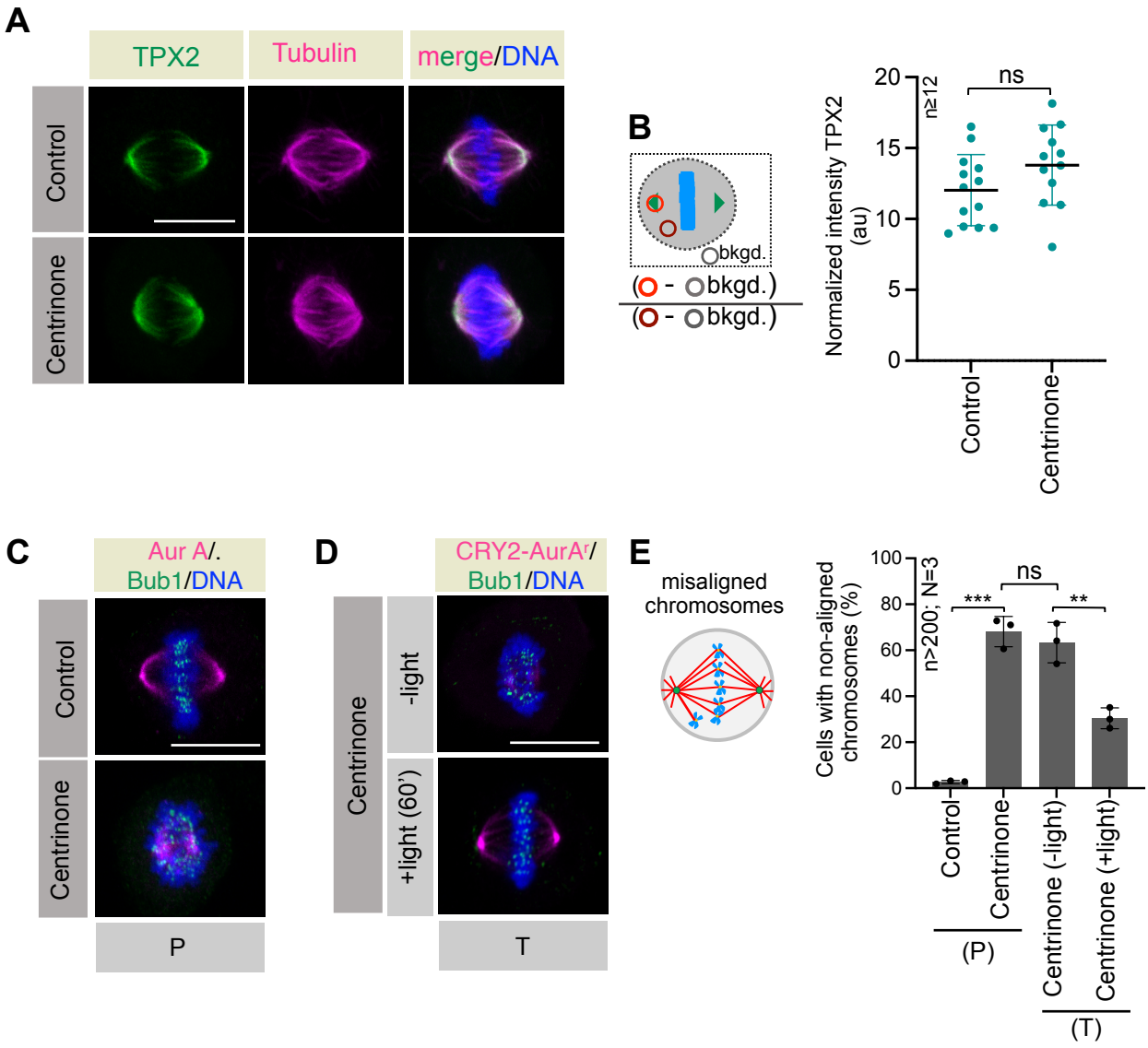
Appendix Fig. S3



Appendix Figure S3

<u>Centrosomal proteins are not enriched at the poles upon Aurora A activation in acentrosomal cells</u>

(A-O) IF analysis of parental (P) (A, D, G, J, M), or transgenic (T) HeLa cells stably coexpressing CRY2-AurA^r and CIB1-MP (B, E, H, K, N) that are either left untreated or treated with Plk4 inhibitor centrinone for 96 h, as indicated. These cells were then stained for different centrosomal components (green) [anti-Cep192 (A, B)/ anti-PCNT (D, E)/ anti-Cdk5Rap2 (G, H)/ anti-Cep152 (J, K)/ anti-γ-tubulin (M, N)] together with anti-tubulin in parental cells or mCherry/SNAP (magenta) in transgenic cells treated with centrinone. DNA is shown in blue. The outcome of the measurement of intensity of centrosomal components in different conditions, as indicated are shown towards the right. Error bars: mean ± SD (n≥10). Exact p-values in each case is determined as follows: Cep192 (control vs centrinone ***p<0.001, CRY2-AurA^r; centrinone (-light vs +light) ***p<0.001, Cep192; centrinone (-light vs +light) p=0.2133 (ns). PCNT (control vs centrinone ***p<0.001, CRY2-AurA^r; centrinone (-light vs +light) ***p<0.001, PCNT; centrinone (-light vs +light) *p=0.0129. Cdk5Rap2 (control vs centrinone ***p<0.001, CRY2-AurA^r; centrinone (-light vs +light) ***p<0.001, Cdk5Rap2; centrinone (-light vs +light) p=0.0935 (ns). Cep152 (control vs centrinone ***p<0.001, CRY2-AurA^r; centrinone (-light vs +light) ***p<0.001, Cep152; centrinone (-light vs +light) p=0.5244 (ns). γ-tubulin (control vs centrinone ***p<0.001, CRY2-AurA^r; centrinone (-light vs +light) ***p<0.001, γ-tubulin; centrinone (-light vs +light) p=0.7617 (ns).
p value is denoted as follows: ns-p≥0.05; *p<0.01; ***p<0.001 as determined by two-tailed unpaired Student's t-test.
Scale bar in (A, B, D, E, G, H, J, K, M, N) represent 10 μm.



Appendix Figure S4

<u>Chromosome alignment errors seen in centrinone-treated acentrosomal cells are significantly rescued by light-induced Aurora A clustering</u>

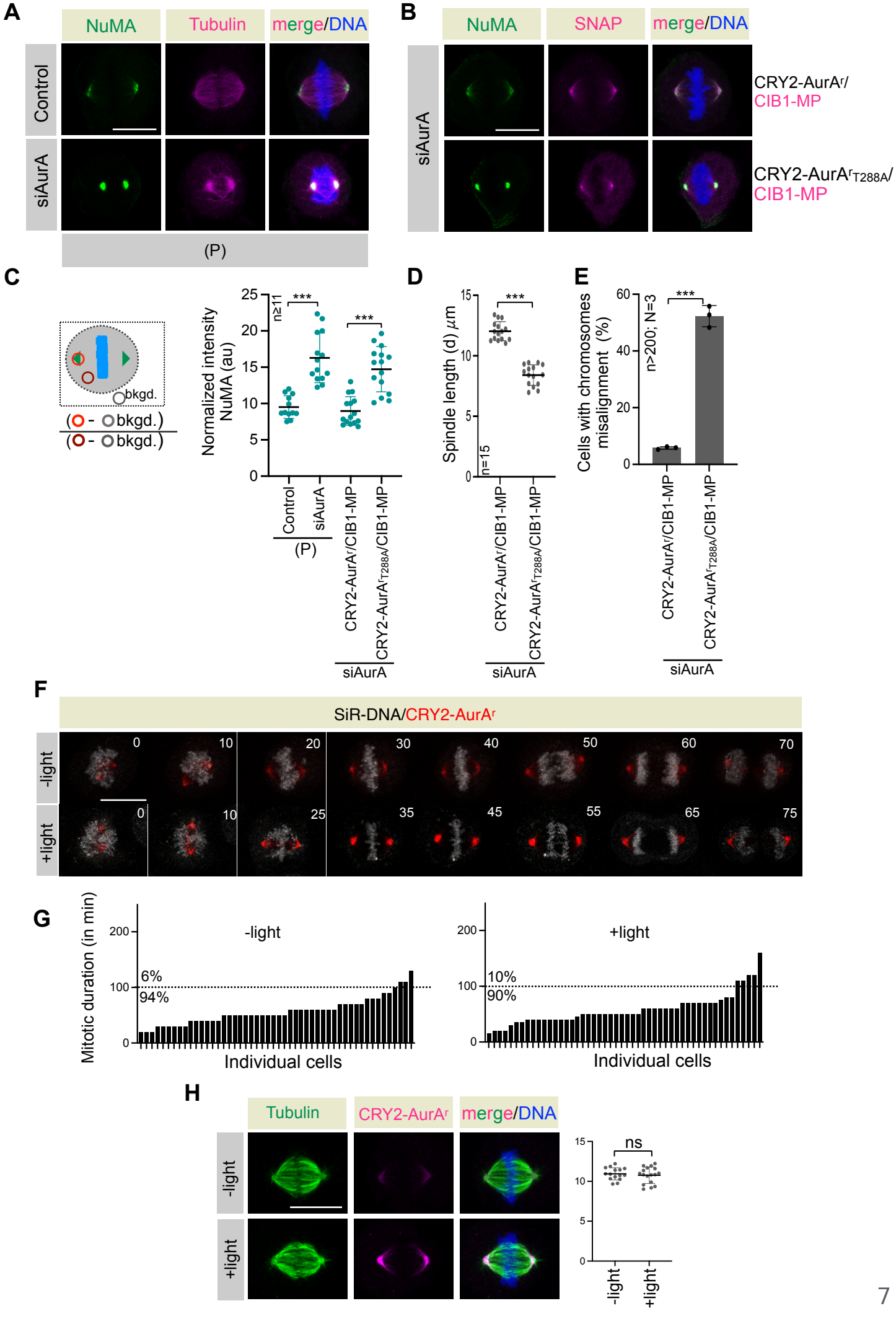
(A, B) IF analysis of parental (P) HeLa cells that were either left untreated or treated with Plk4 inhibitor-centrinone for 96 h. These cells were then stained with either anti-TPX2 (AurA) (green) and anti-α-tubulin antibodies, as indicated. DNA is shown in blue (A). In such conditions, centrosomal TPX2 intensity was assessed and quantified in (B). Error bars: mean ± SD (n≥12). Exact p values is ns-p=0.110.

(C-E) IF analysis of parental (P) (C), or transgenic (T) HeLa cells stably coexpressing CRY2-AurAr and CIB1-MP (D) that are either left untreated or treated with Plk4 inhibitor-centrinone for 96 h, and also exposed to blue light, as indicated. These cells were then stained with either anti-Aurora A (AurA) (magenta) or anti-mCherry antibodies, together with anti-Bub1, as indicated. DNA is shown in blue. In such conditions, chromosome misalignments were assessed and quantified in (E). Error bars: mean ± SD (n≥200 from 3 different experiments). Exact p values from left to right are ***p<0.001; ns-p=0.4918; **p=0.0045.

p value is denoted as follows: ns-p≥0.05; **p<0.01; ***p<0.001 as determined by two-tailed unpaired Student's t-test.

Scale bar in (A, C, D) represent 10 μm.

Appendix Fig. S5



Appendix Figure S5
Light-induced Aurora A clustering approach do not impact mitotic duration

(A) IF analysis of parental (P) HeLa cells treated with control or AurA siRNA for 60 h and stained for anti-NuMA (green) and anti-β-tubulin (magenta). DNA is shown in blue. The intensity of NuMA in control and siRNA-treated samples is measured and plotted below in (C).
(B, C) IF analysis of HeLa cells stably coexpressing either CRY2-AurA^{r} and CIB1-MP or CRY2-AurA$^{r}_{T288A}$ and CIB1-MP, depleted for endogenous Aurora A, and exposed to blue light. These cells were then stained with anti-NuMA (green) and anti-SNAP (magenta) antibodies (B). Please note that anti-SNAP antibodies were used to visualize CIB1-MP, and thus to follow CRY2-AurA^{r} (wild-type and mutant protein). DNA is shown in blue. The intensity of NuMA in the specified conditions is measured in 'C'. Note the enrichment of NuMA at the spindle poles in siAurA-treated parental control, or in cells expressing phosphodead- CRY2-AurA$^{r}_{T288A}$ allele of Aurora A. Also note the rescue in NuMA intensity at the spindle poles in cells expressing wild-type allele of CRY2-AurA^{r}. Error bars: mean ± SD (n≥11). Exact p value is ***p<0.001 and ***p<0.001 from left to right.
(D, E) Measurement of spindle length (D), or chromosome misalignments (E) in transgenic HeLa cells stably expressing either CRY2-AurA^{r} and CIB1-MP or CRY2-AurA$^{r}_{T288A}$ and CIB1-MP, depleted for endogenous Aurora A, and exposed to blue light. Cells were stained with anti-β-tubulin antibodies, and the DNA was labelled with Hoechst 33342. Error bars represent mean ± SD in D (n = 15 cells). Exact p-values is ***p < 0.001. Error bars represent mean ± SD in E (n>200; N=3). Exact p-values is ***p < 0.001.
(F, G) Representative confocal live-cell images of cells coexpressing CRY2-AurA^{r} and CIB1-MP, which were either not exposed to blue light or exposed to blue light, as indicated (F). Quantification of mitotic duration from the live-cell imaging experiments shown in (G). Each horizontal bar represents an individual cell (n > 50 cells per condition).
(H) IF analysis of transgenic HeLa cells coexpressing CRY2-AurA^{r} and CIB1-MP that were either maintained in the dark (−light) or exposed to blue light (+light). Spindle length was quantified as the distance between the two spindle poles. Cells were stained with anti-β-tubulin (green) and anti-mCherry (magenta) antibodies to detect CRY2-AurA^{r}. DNA is shown in blue. Quantification of spindle length is shown on the right. Error bars represent mean ± SD (n ≥ 15 cells). Exact *p* value: ns-p=0.5711.
p-value is denoted as ***p<0.001, as determined by two-tailed unpaired Student's t-test. Scale bars in (A, B, F, H) represent 10 μm.