Supplemental Information

Supplemental Figure 1

Tpr down-modulation caused chromosome lagging or bending often disrupted p53 centrosomal localization

Exemplary confocal images of immunofluorescence analysis of mitotic HeLa cells transfected with control or Tpr siRNA and analyzed 72 h post-transfection. The cells were analyzed by immunofluorescence using antibodies against with Tpr (rabbit anti-Tpr, sc-101294, from Santa Cruz Biotechnology) and p53 (mouse anti-p53, DO-7 from Dako). Alexa Fluor 488 goat anti-mouse IgG (A11029) and Rhodamine Red-X goat anti-rabbit IgG (R6394) (from Invitrogen) was used as secondary antibody. DNA was counterstained using DAPI (Invitrogen).

Supplemental Figure 2

A subpopulation of Tpr is localized at the centrosome during mitosis.

(A) Immunoprecipitation of extracts from mitotic HeLa cells incubated with anti-γ-tubulin (XXX from Sigma-Aldrich) or nonspecific rabbit antibodies (IgG) and then analyzed by SDS-PAGE, followed by immunoblotting with anti-Tpr (mouse anti-Tpr, sc-67116, from Santa Cruz Biotechnology) antibodies.

(B) Flow chart for Tpr centrosomal staining protocol, permeabilization with triton X before fixation.

(C) Confocal microscopy images of HeLa cells at different cell cycle stages, stained with anti-γ-tubulin (XXX from Sigma-Aldrich) (green) and anti-Tpr (from Gerace Lab in Scripps Research Institute) (red) antibodies. Goat anti-mouse Alexa Fluor-488 or rabbit Rodamine (both from Invitrogen) were used as secondary antibodies. Chromatin was stained with DAPI (blue).

Supplemental Figure 3

Transient overexpression of Tpr or its fragments did not alter Aurora-A centrosomal localization.

(A) Scheme of full-length Tpr and three mutants with GFP tags. Numbers on the left refer to amino acids.

(B) Exemplary confocal images of mitotic HeLa cells transfected with plasmids overexpressing GFP-Tpr (full length), GFP-Tpr-N, GFP-Tpr-M and GFP-Tpr-C respectively. 48 h after transfection, cells were fixed, stained with

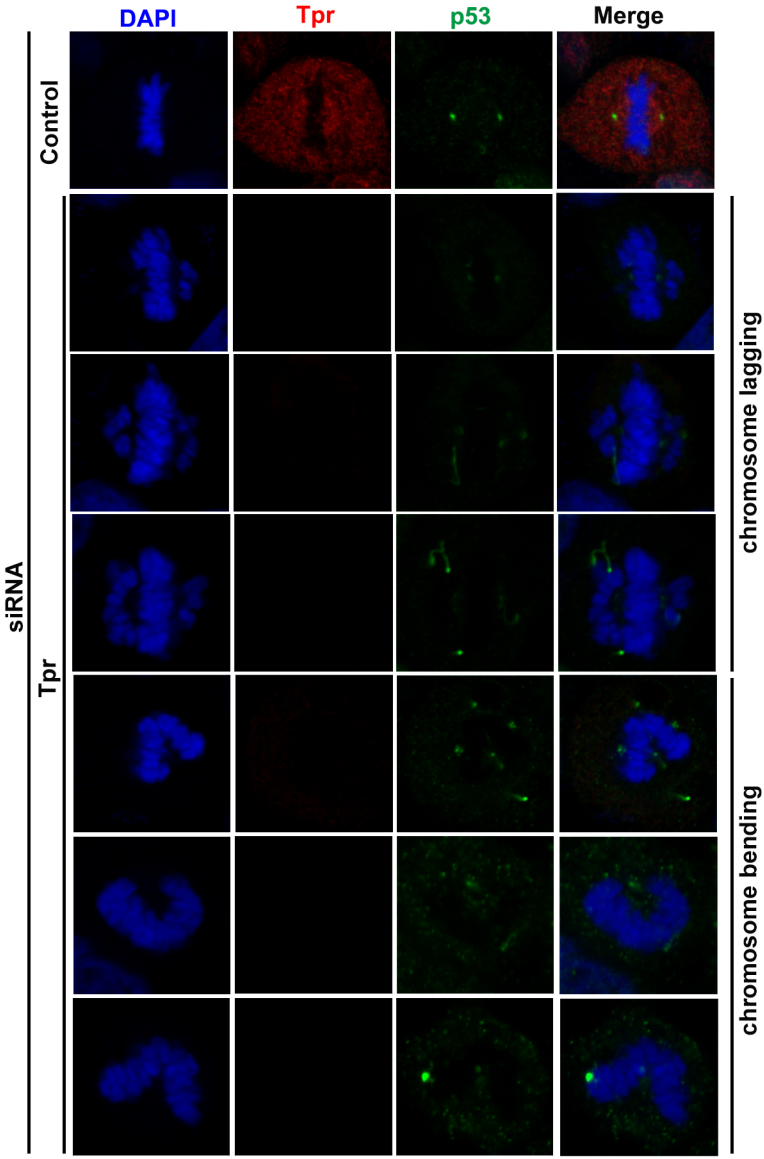
anti-Aurora A antibodies (red) and GFP (rabbit, Invitrogen) antibodies (green), and analyzed by confocal laser microscopy. Chromatin was stained with DAPI (blue).

(C) Other representative images together with non-transfected cells.

Supplemental Figure 4

A subpopulation of Tpr depletion cells exhibited chromosome segregation defects (white arrow heads indicated long chromosome lagging fibers) while some Aurora A still partially localized on the centrosomes.

HeLa cells transfected with control or Tpr siRNA and analyzed 72 h post-transfection; the cells were stained with anti-Aurora (green) and anti-Tpr (red) antibodies and DAPI (blue).



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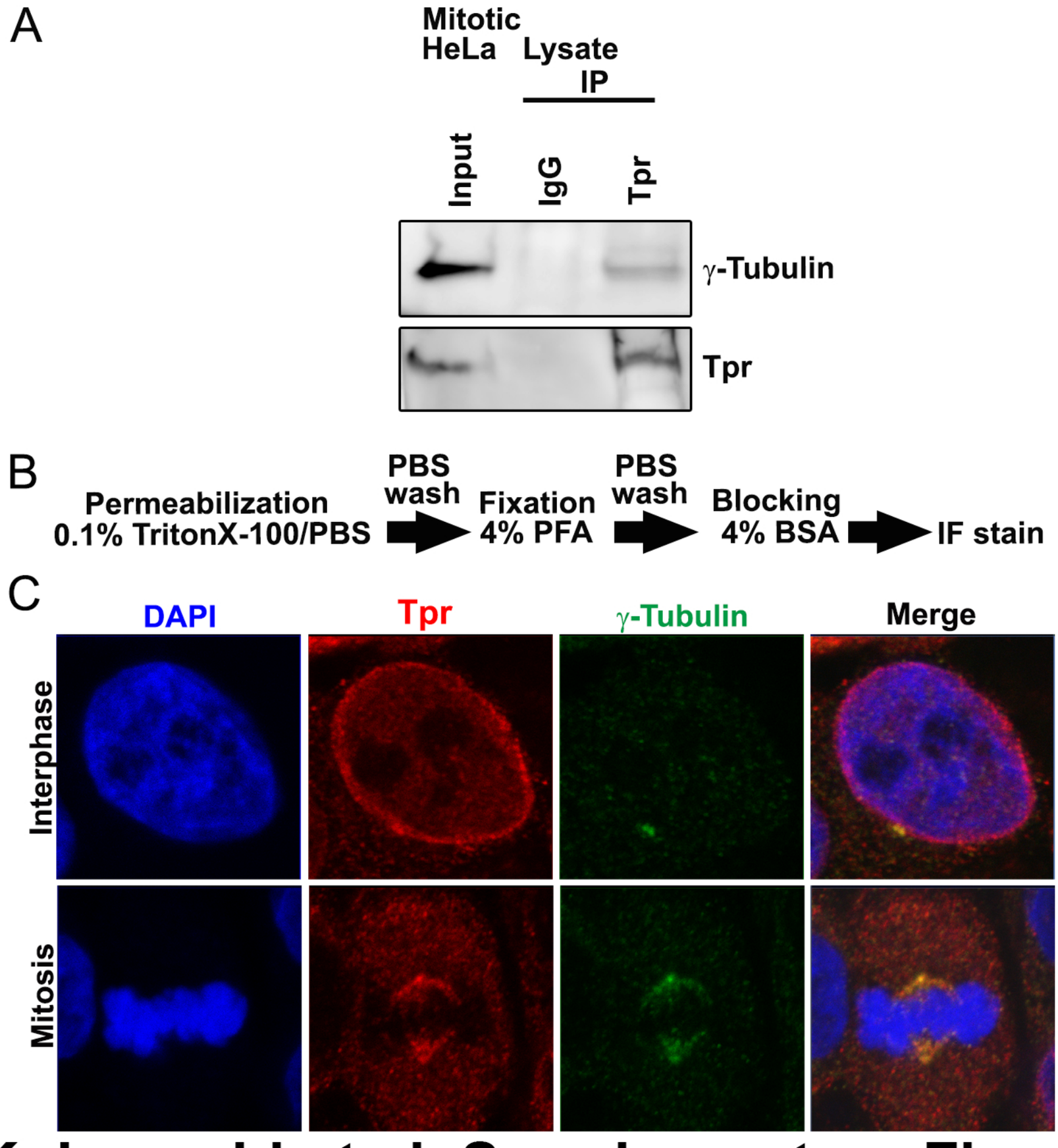
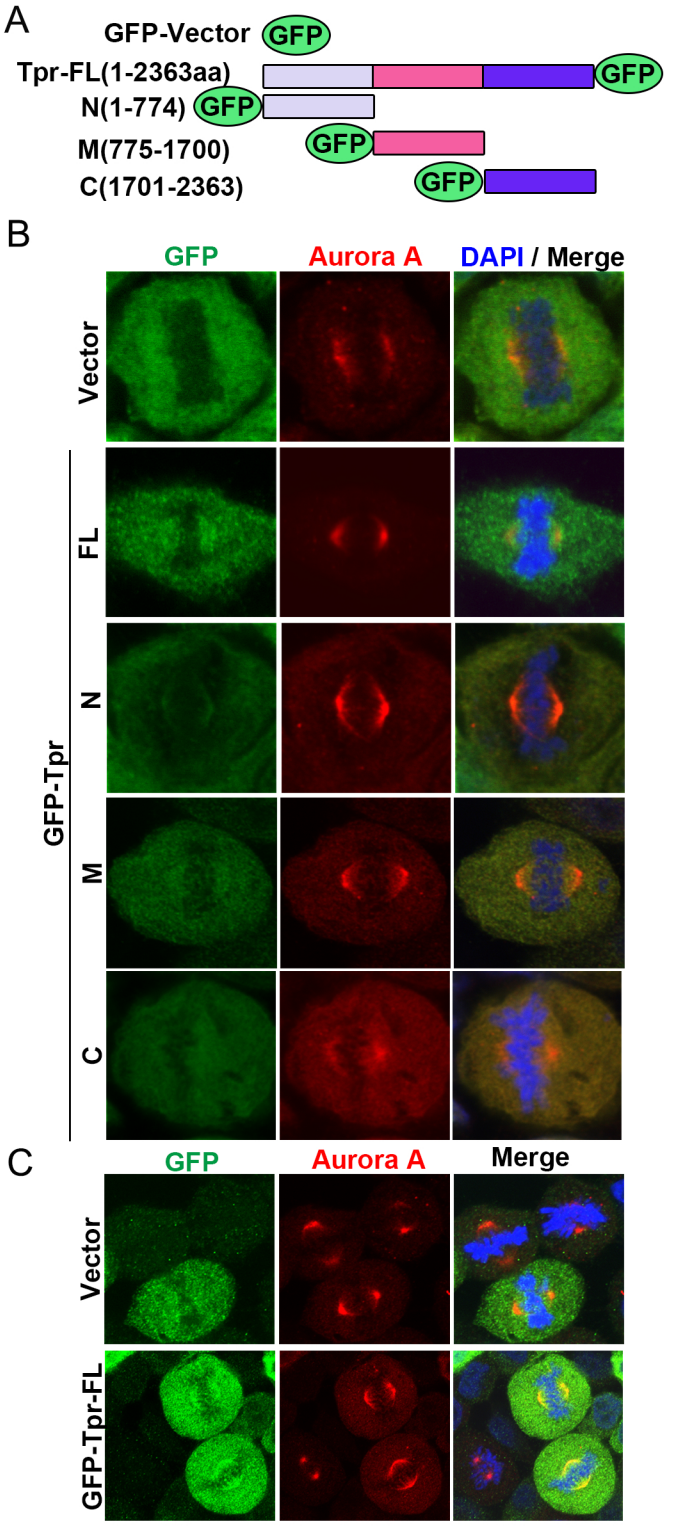


Kobayashi et.al. Supplementary Figure 2.



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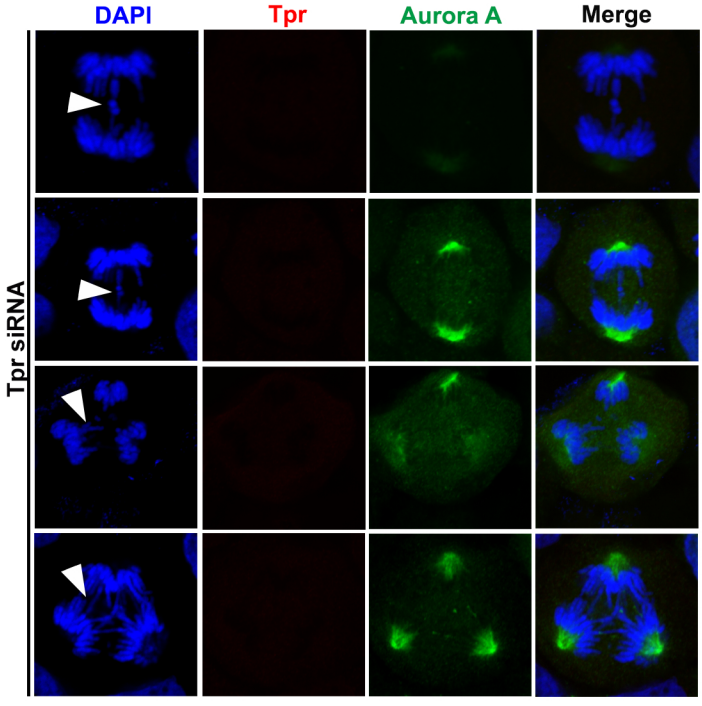

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