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Supplemental Information

The Aurora-A/TPX2 Axis Directs Spindle Orientation in Adherent Human Cells by Regulating NuMA and Microtubule Stability

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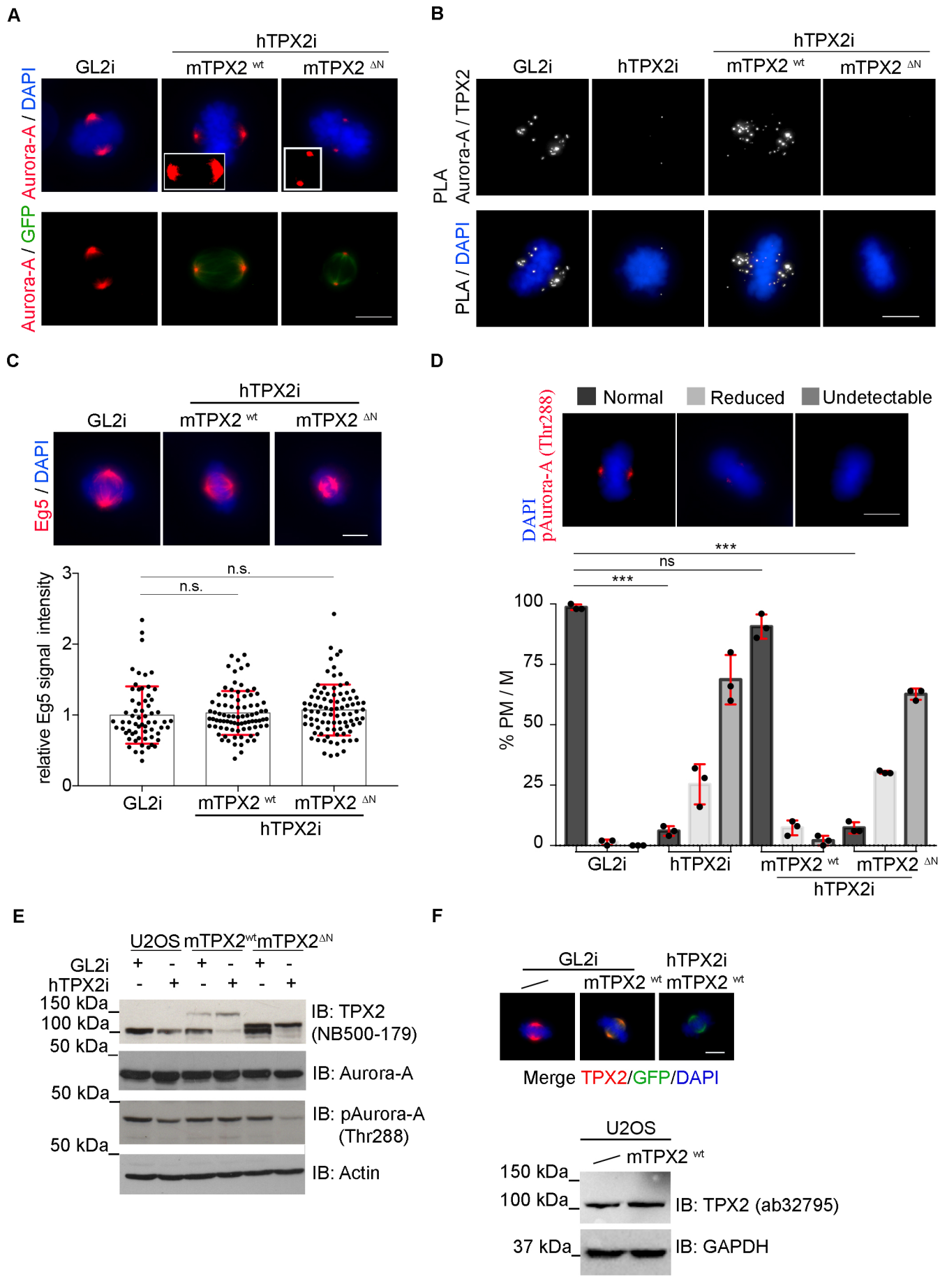
A
hTPX2i
GL2i
mTPX2 wt
mTPX2 ΔN
Aurora-A / DAPI
Aurora-A / GFP
B
hTPX2i
GL2i
hTPX2i
mTPX2 wt
mTPX2 ΔN
PLA Aurora-A / TPX2
PLA / DAPI
C
hTPX2i
GL2i
mTPX2 wt
mTPX2 ΔN
Eg5 / DAPI
relative Eg5 signal intensity
n.s.
n.s.
GL2i
mTPX2 wt
mTPX2 ΔN
hTPX2i
D
Normal
Reduced
Undetectable
DAPI
pAurora-A (Thr288)
***
ns
***
% PM / M
GL2i
hTPX2i
mTPX2 wt
mTPX2 ΔN
hTPX2i
E
U2OS mTPX2wt mTPX2ΔN
GL2i
hTPX2i
150 kDa
100 kDa
50 kDa
IB: TPX2 (NB500-179)
IB: Aurora-A
IB: pAurora-A (Thr288)
IB: Actin
F
GL2i
hTPX2i
mTPX2 wt
mTPX2 wt
Merge TPX2/GFP/DAPI
U2OS
mTPX2 wt
150 kDa
100 kDa
IB: TPX2 (ab32795)
37 kDa
IB: GAPDH

Figure S1. Characterization of Aurora-A and Eg5 in mTPX2^{wt} or mTPX2$^{\Delta N}$ U2OS expressing cells. Related to Figure 1. A. IF panels show the localization of GFP-mTPX2 (green) and Aurora-A (red) in metaphase, in the indicated conditions. hTPX2i indicates RNAi-mediated depletion of endogenous TPX2, while GL2i indicates the control condition. Insets show uniformly enhanced Aurora-A signals to better highlight that Aurora-A in hTPX2i/mTPX2$^{\Delta N}$ metaphases is centrosomal only. **B.** Images show the Aurora-A/TPX2 *is*PLA signals in the indicated cell lines. The specific antibodies used for *is*PLA reactions are defined in the Star Methods section and Key Resources Table. **C.** Values of Eg5 signal intensity (normalized to the average control GL2i value in each experiment) at prometa and metaphase (PM/M) spindles under the indicated conditions are shown in the dot plot; exemplifying images are shown in the IF panels. **D.** Histograms represent the percentage of PM/M displaying normal (example of a GL2i metaphase shown in the IF panels), reduced or undetectable (examples of hTPX2i/mTPX2$^{\Delta N}$ metaphases shown in the IF panels) p-Thr288-Aurora-A signal at spindle poles under the indicated conditions. **E.** Immunoblotting with the indicated antibodies and cell lines, in GL2i or hTPX2i conditions. Molecular weights are on the left. Cultures were harvested 30 h after RNAi and enriched in prometaphase by monastrol treatment (16 h; 100 μM). **F.** The anti-TPX2 antibody raised in mouse (Abcam, Ab32795) does not recognize the exogenously expressed GFP-mTPX2. By western blotting analysis a single band corresponding to endogenous TPX2 appears in both cell lines (control and overexpressing one). IF panels above show that TPX2 IF signals are evident only in GL2i conditions (left and central panels), where endogenous TPX2 is present, while they are not detected after RNAi (right panel), when only the exogenous GFP-mTPX2 remains (green channel). At least 60 (C) and 150 (D) PM/M were analyzed for each condition from 3 replicate experiments. Error bars: SD; n.s., not significant, *** $p < 0.0001$; Kruskal-Wallis Anova (C) or chi-square (and Fisher's exact; D) test. Scale bars, 10 μm.

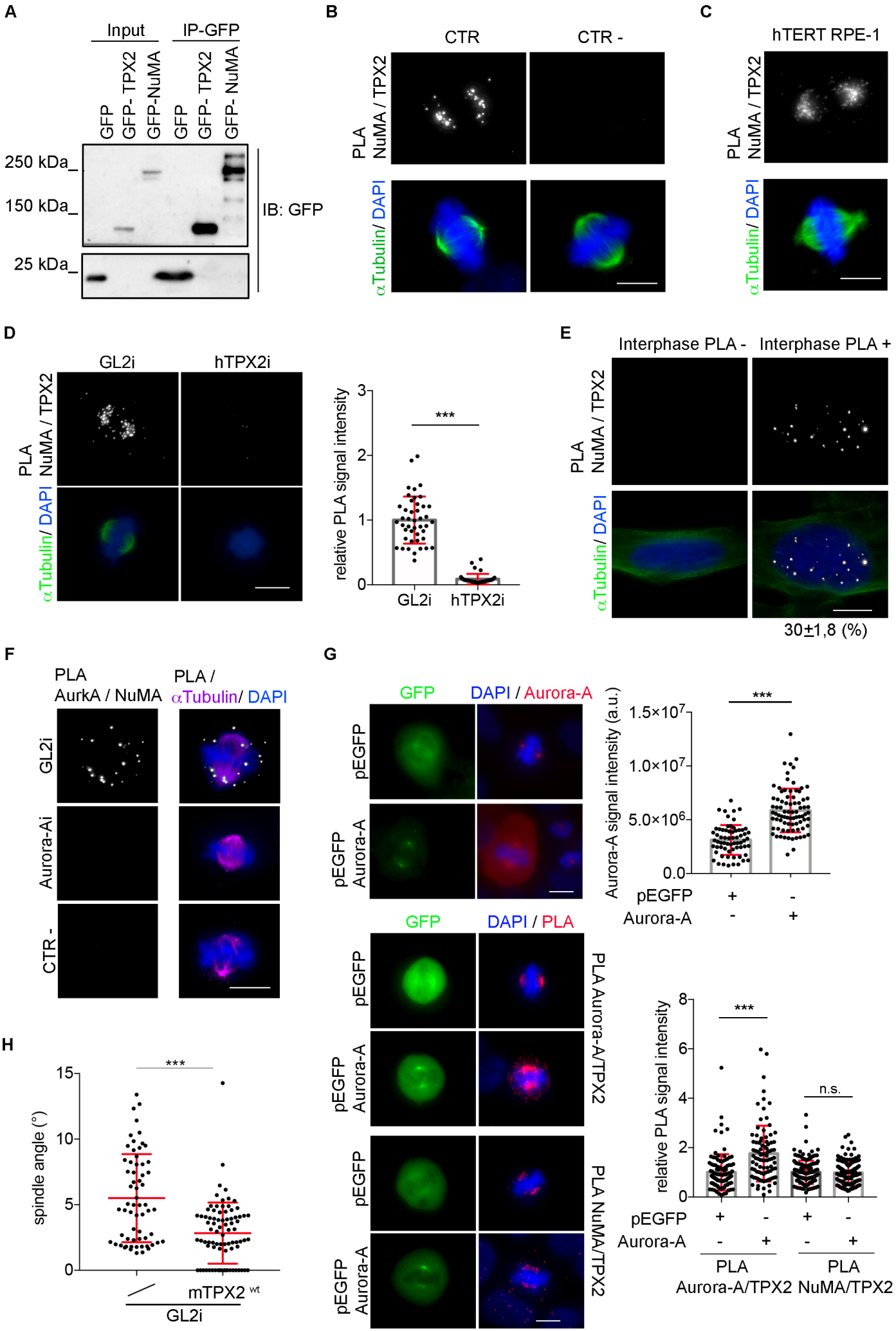
A
Input
IP-GFP
GFP
GFP- TPX2
GFP-NuMA
GFP
GFP- TPX2
GFP- NuMA
250 kDa_
150 kDa_
25 kDa_
IB: GFP
B
CTR
CTR -
PLA NuMA / TPX2
αTubulin/ DAPI
C
hTERT RPE-1
PLA NuMA / TPX2
αTubulin/ DAPI
D
GL2i
hTPX2i
PLA NuMA / TPX2
αTubulin/ DAPI
relative PLA signal intensity
***
GL2i
hTPX2i
E
Interphase PLA -
Interphase PLA +
PLA NuMA / TPX2
αTubulin/ DAPI
30±1,8 (%)
F
PLA AurkA / NuMA
PLA / αTubulin/ DAPI
GL2i
Aurora-Ai
CTR -
G
GFP
DAPI / Aurora-A
pEGFP
pEGFP Aurora-A
Aurora-A signal intensity (a.u.)
***
pEGFP
Aurora-A
GFP
DAPI / PLA
pEGFP
pEGFP Aurora-A
PLA Aurora-A/TPX2
PLA NuMA/TPX2
relative PLA signal intensity
***
n.s.
pEGFP
Aurora-A
PLA Aurora-A/TPX2
PLA NuMA/TPX2
H
spindle angle (°)
***
mTPX2 wt
GL2i

Figure S2. Interactions between NuMA and TPX2 or NuMA and Aurora-A under different experimental conditions. Related to Figure 2. A. Immunoblotting using the GFP antibody on total lysates and GFP-Trap immunoprecipitates under the indicated conditions, to compare the levels of GFP alone (membrane as in main Figure 2A) and exogenous GFP-tagged proteins (samples as in main Figure 2A). **B-E.** IF and *is*PLA panels show NuMA/TPX2 interaction in U2OS mitoses (B, D) or interphase cells (E; % and SD from 3 independent experiments are indicated; about 1500 total counted cells) and hTERT-RPE-1 mitotic cells (C). Loss of the signal in the presence of one primary antibody only (CTR-) (B), or following TPX2 RNAi (D) is shown. Values of the *is*PLA signals in (D) were quantified and normalized to the average control (GL2i) value in each experiment. **F.** *is*PLA signals corresponding to the Aurora-A/NuMA interaction in U2OS cells are lost upon Aurora-A RNAi, or in the presence of one primary antibody only (CTR-). **G.** *is*PLA images (right column; merged with DAPI) in GFP or GFP-Aurora-A (left column) transfected U2OS cells are shown in the middle and lower panels, while upper ones show Aurora-A IF signals. IF and *is*PLA signals are quantified in the dot plots on the right (*is*PLA signals are normalized to the average control pEGFP value in each experiment). **H.** Spindle angle values of GL2-interfered - control (/; samples as in Figure 1) or $mTPX2^{wt}$ expressing - U2OS metaphase cells (see Figure 1 and Supplementary Figure 1) are shown in the dot plot. Analyzed cells for each condition from 2-3 replicate experiments were: 40-60 prometa-metaphases (D) or >60 prometa-metaphases (G) or metaphases (H). Specific antibodies pairs used for each *is*PLA reaction are defined in the Star Methods section Key Resources Table. a.u., arbitrary units. Error bars: SD; n.s., not significant; *** $p < 0.0001$; Mann-Whitney test. Scale bars, 10 μm.

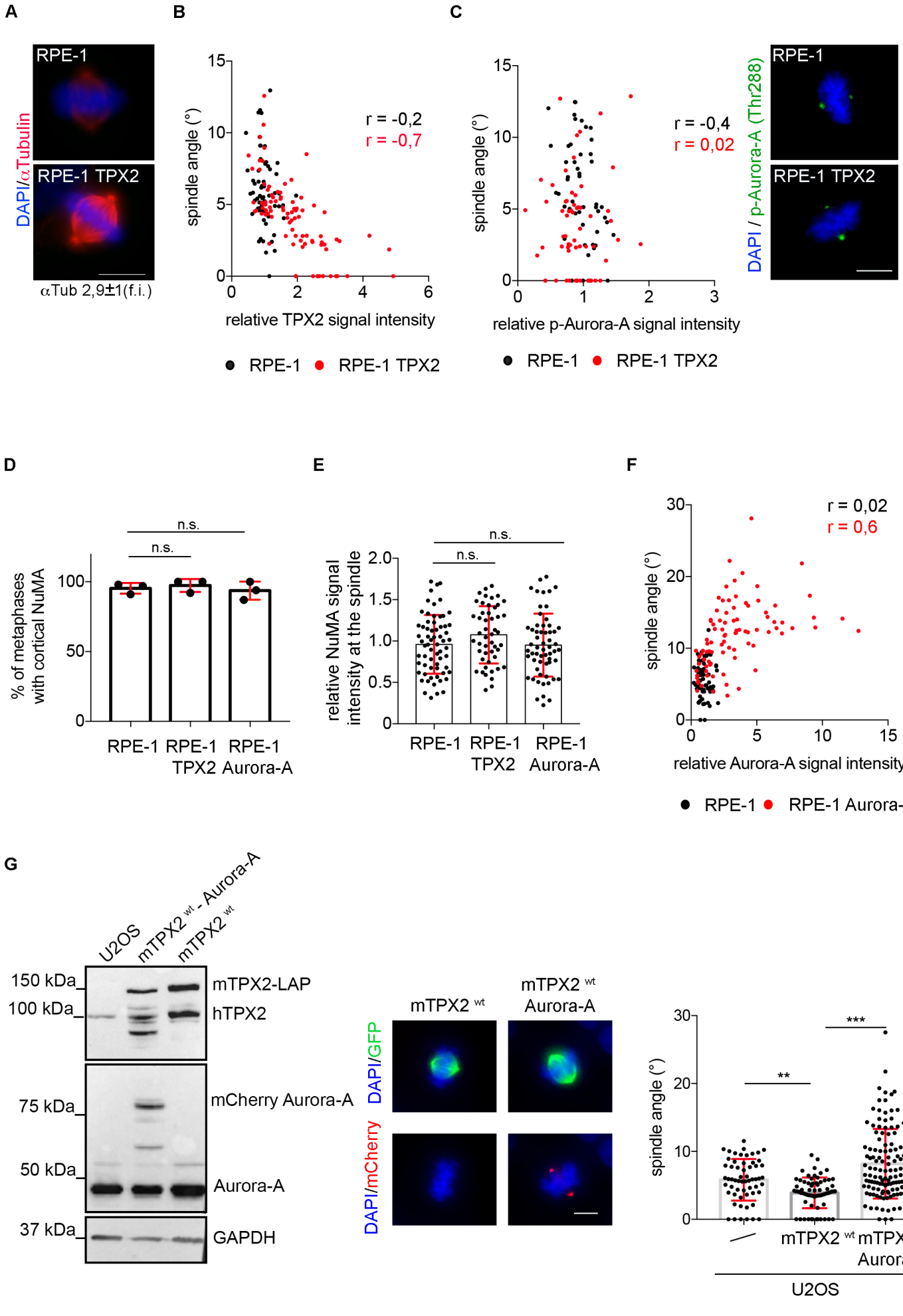
A
RPE-1
RPE-1 TPX2
DAPI/αTubulin
αTub 2,9±1(f.i.)
B
spindle angle (°)
relative TPX2 signal intensity
r = -0,2
r = -0,7
RPE-1
RPE-1 TPX2
C
spindle angle (°)
relative p-Aurora-A signal intensity
r = -0,4
r = 0,02
RPE-1
RPE-1 TPX2
DAPI / p-Aurora-A (Thr288)
D
% of metaphases with cortical NuMA
n.s.
n.s.
RPE-1
RPE-1 TPX2
RPE-1 Aurora-A
E
relative NuMA signal intensity at the spindle
n.s.
n.s.
RPE-1
RPE-1 TPX2
RPE-1 Aurora-A
F
spindle angle (°)
relative Aurora-A signal intensity
r = 0,02
r = 0,6
RPE-1
RPE-1 Aurora-A
G
U2OS
mTPX2 wt - Aurora-A
mTPX2 wt
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
mTPX2-LAP
hTPX2
mCherry Aurora-A
Aurora-A
GAPDH
mTPX2 wt
mTPX2 wt Aurora-A
DAPI/GFP
DAPI/mCherry
spindle angle (°)
**
***
mTPX2 wt
mTPX2 wt Aurora-A
U2OS

Figure S3. NuMA localization, α-tubulin levels and spindle angle analyses in Aurora-A and/or TPX2 overexpressing cells. Related to Figures 3 and 4. A. The α-tubulin signal (red; average fold increase compared to controls is indicated) in the hTERT-RPE-1 cell line overexpressing TPX2 is shown. Scatter plots represent TPX2 (**B**) or p-Thr288-Aurora-A (**C**) signal intensity at metaphase spindles or spindle poles respectively (conservative fixation), against the spindle angle values for each single cell, under the indicated conditions. Each fluorescence intensity value in C is the average value between the two spindle poles signals within one cell; representative metaphases are shown in the IF panels. **D.** Histograms represent the percentage of metaphases displaying NuMA at the cell cortex under the indicated conditions, while NuMA signal intensity values at spindles are shown in the dot plot in **E**. Scatter plots in **F.** represent Aurora-A signal intensity in the whole cell, against the corresponding spindle angle values, under the indicated conditions. FLAG-tagged TPX2-overexpressing hTERT-RPE-1 cultures and MYC-tagged Aurora-A overexpressing ones are indicated as RPE-1 TPX2 and RPE-1 Aurora-A throughout the Figure, while non-induced controls are indicated as RPE-1. Fluorescence intensity values are normalized to the average control one (RPE-1) in each experiment. **G.** Western blotting analysis using anti-Aurora-A and anti-TPX2 antibodies in the indicated cell lines; GAPDH is used as normalizing control; molecular weights are on the left. IF central panels show representative metaphases from the cell lines overexpressing TPX2 only (GFP, green) or together with Aurora-A (mCherry, red). The dot plots on the right indicate corresponding spindle angle values.

For each condition, at least 50 metaphases from 3 replicate experiments were analyzed; scored metaphases in D were 75-150 per condition. Error bars: SD; n.s., not significant, ** $p < 0.001$; *** $p < 0.0001$; chi-square (and Fisher's exact; D) or Kruskal-Wallis Anova (E, G) test. r, correlation values are indicated (B, C, F). Scale bars, 10 μm.

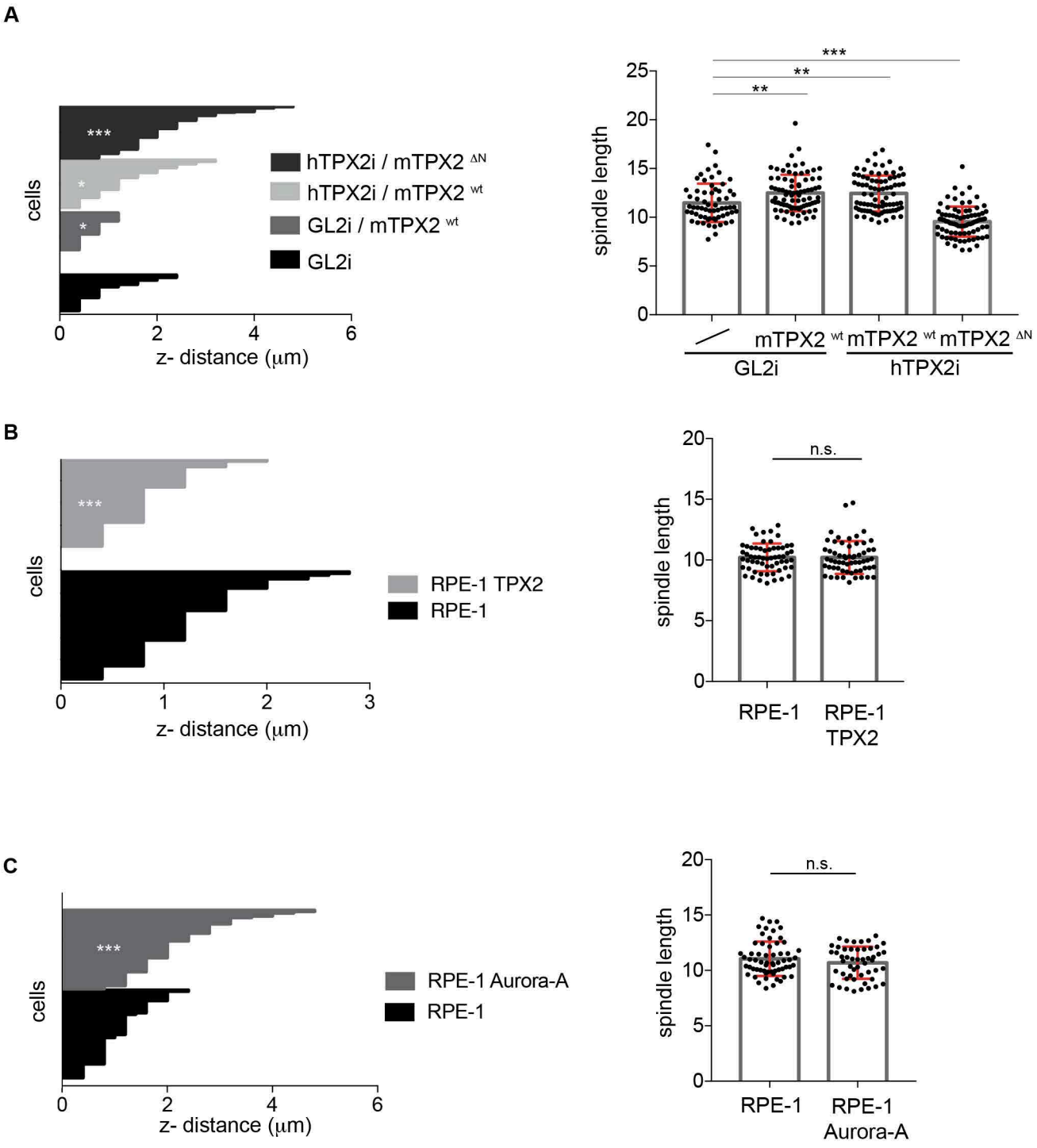

Figure S4. Values used for spindle angle measures. Related to Figures 1, 3, 4. The single measures of pole-to-pole distances along the z-axis (left column graphs) and on the xy axis (defined "spindle length", from Maximum Intensity Projections images, right column graphs), used for the main angle measures in Figures 1, 3, 4 and S2 are shown. Note that under conditions where spindle angle variations were reported (main Figures) pole-to-pole distance along the z-axis is always altered. FLAG-tagged TPX2-overexpressing cultures and MYC-tagged Aurora-A overexpressing one are indicated as RPE-1 TPX2 and RPE-1 Aurora-A throughout the Figure, while non-induced controls are indicated as RPE-1. Sample sizes as in corresponding main Figures 1, 3, 4 and S2. Error bars: SD; n.s., not significant, * $p<0.01$, ** $p < 0.001$, *** $p < 0.0001$; t-test or Mann-Whitney test. The reference condition is "GL2i" or "RPE-1".