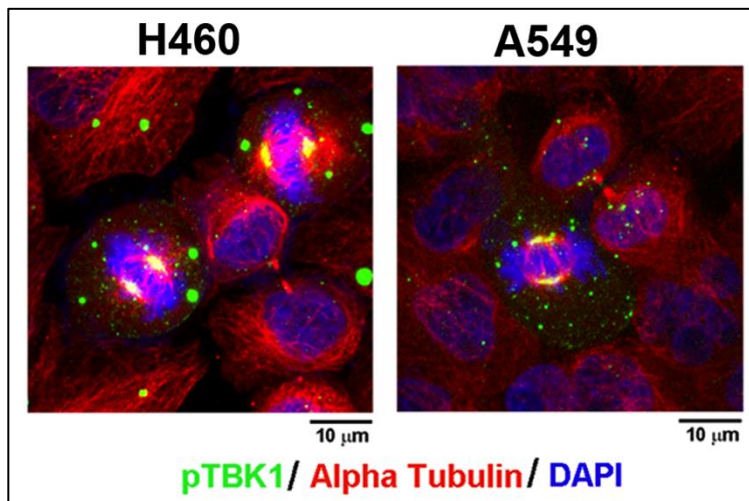


**Supplementary Figure 1. Phospho-TBK1 localizes to centrosomes and mitotic spindles non-small cell lung cancer cell lines as well as immune cells during mitosis.** (a) Confocal microscopy images of Calu-6 cells at prometaphase, metaphase and telophase/cytokinesis stained for phospho-TBK1 (green), alpha tubulin (red) and DNA (DAPI, blue), scale bar=10  $\mu$ m

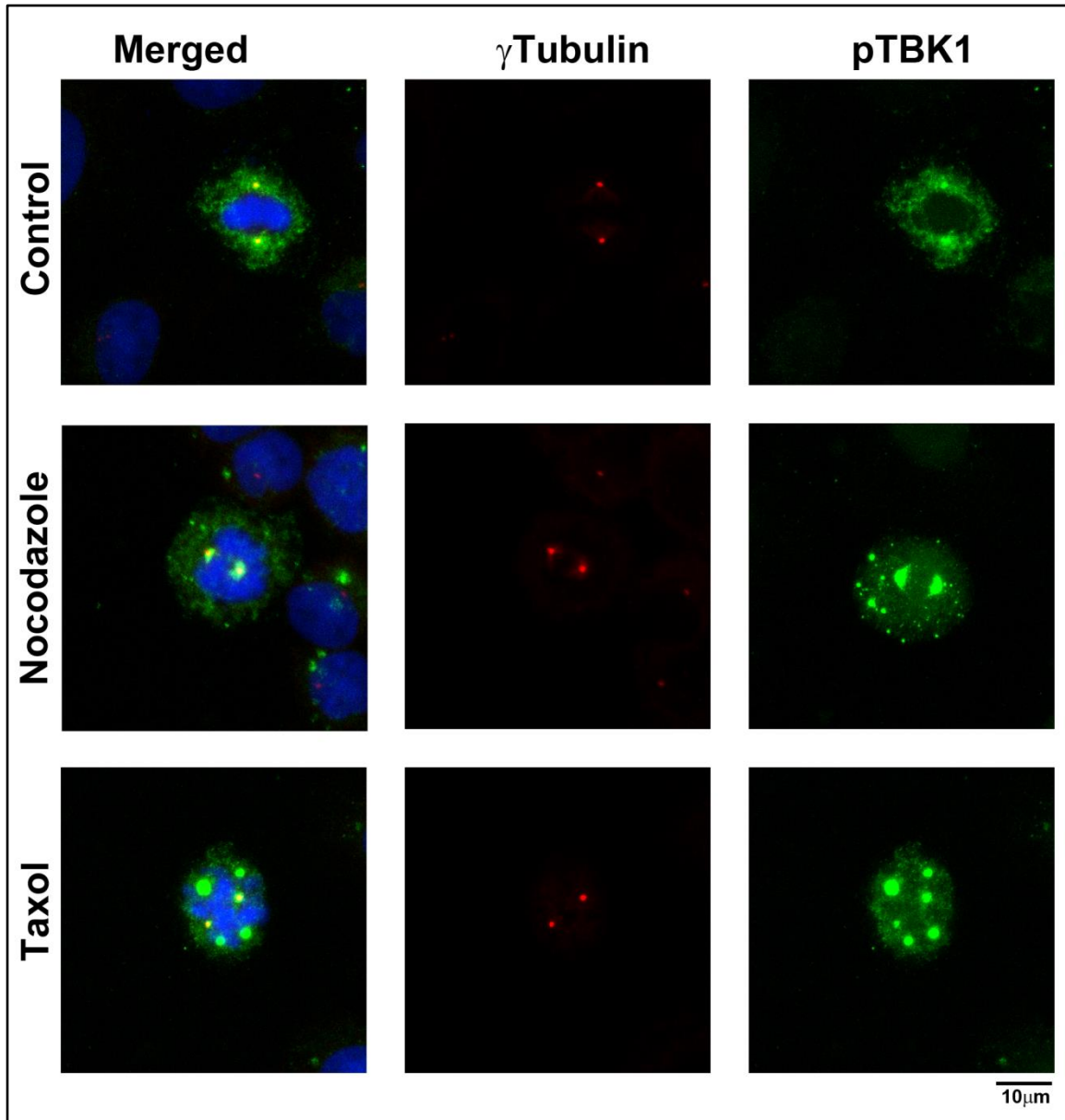
(b) Confocal microscopy images of PC9 cells at prophase, metaphase, metaphase, telophase/cytokinesis and non-mitotic cells stained for phospho-TBK1 (green), alpha tubulin (red) and DNA (DAPI, blue), scale bar=10  $\mu$ m. (c) Confocal image taken at a lower magnification showing the levels of phospho-TBK1 in mitotic as well as non-mitotic PC9 cells stained for phospho-TBK1 and alpha tubulin scale bar=25  $\mu$ m. (d) AALE cells stained for phospho-TBK1 and CEP170 scale bar=25 $\mu$ m.

(e) U937 (histiocytic lymphoma cells) and Daudi (Burkitt's lymphoma; B lymphoblast) cells stained for phospho-TBK1 (green), alpha tubulin (red) and DNA (DAPI, blue), scale bar=10  $\mu$ m.



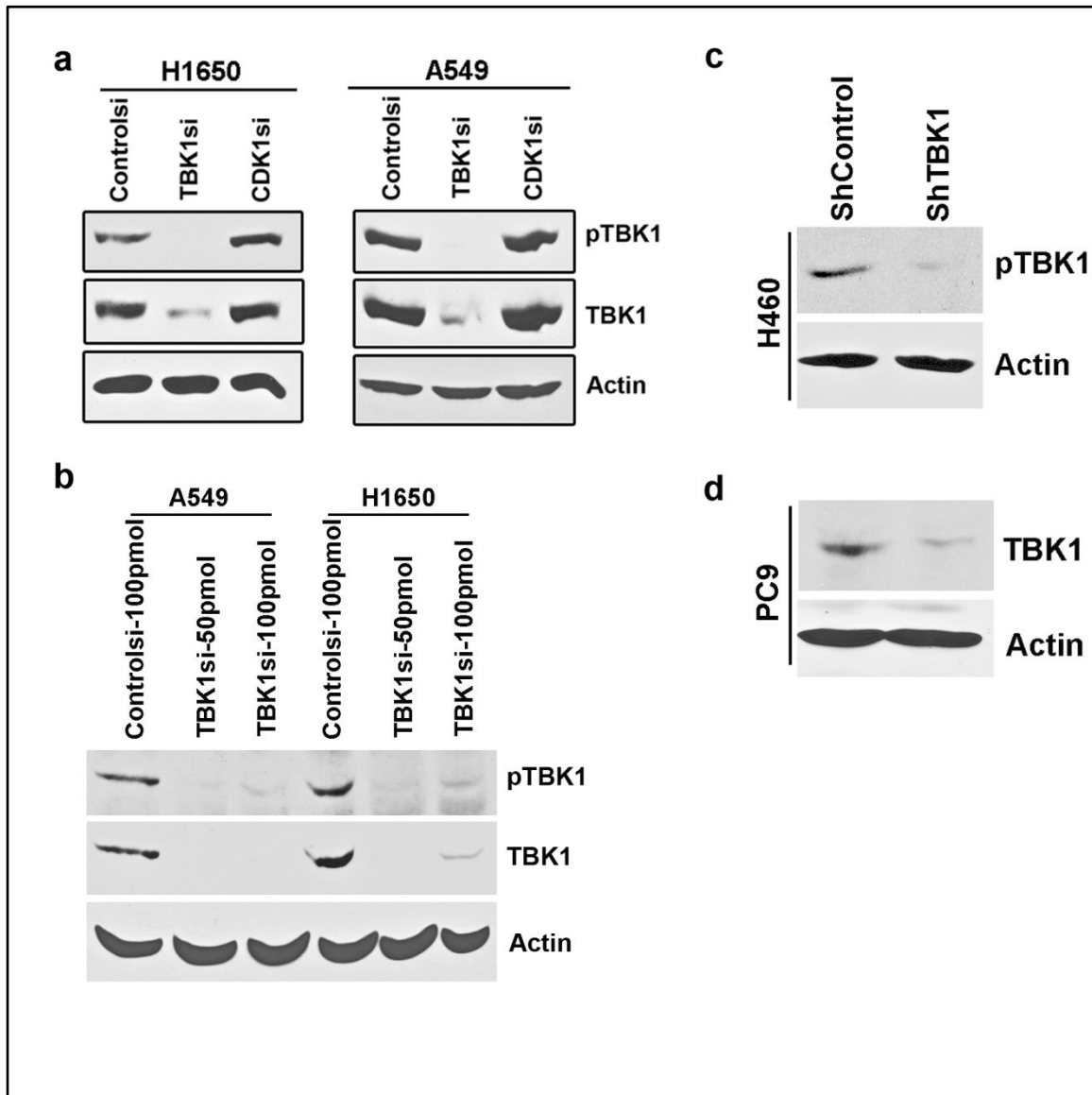
**Supplementary Figure 2. Centrosomal localization of phospho-TBK1 as detected by a second antibody**

Detection of pTBK1 by immunofluorescence using a second pTBK1 antibody from a different source (Origene) yielded similar results. Phospho-TBK1 (green), alpha tubulin (red) and DNA (DAPI, Blue). This experiment confirms that the centrosomal localization of phospho-TBK1 can be detected using multiple antibodies from different sources.



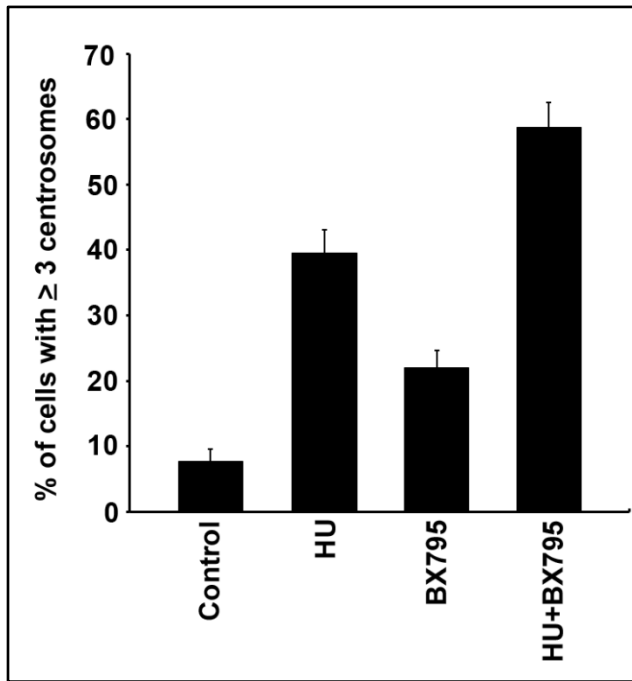
**Supplementary Figure 3. Centrosomal localization of TBK1 does not depend on microtubule integrity**

Confocal microscopy images of H460 cells treated with nocodazole or taxol demonstrating that pTBK1 localizes to centrosomes when MTs are hyperstabilized by taxol or depolymerized by nocodazole.



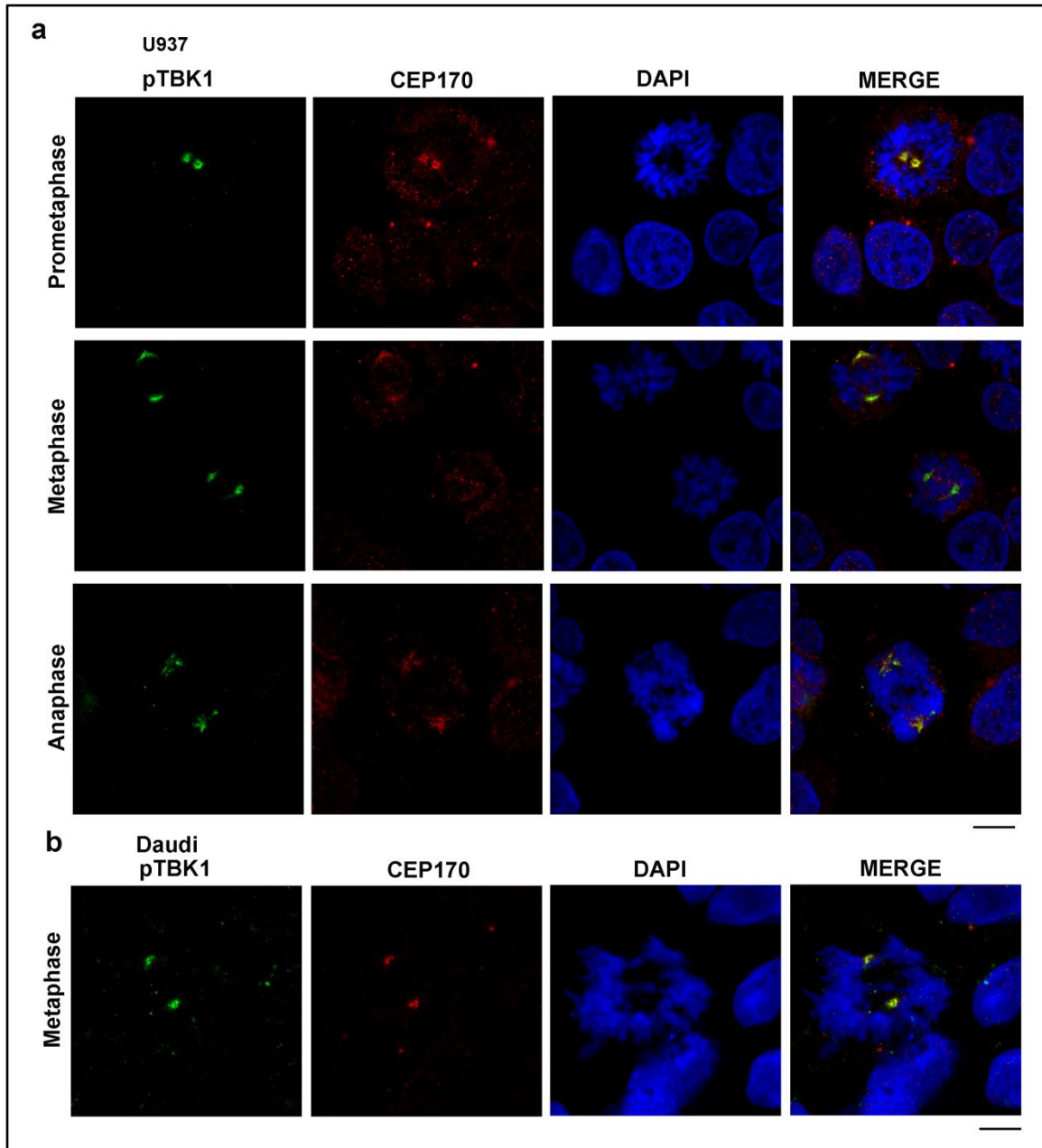
**Supplementary Figure 4. Depletion of TBK1 by siRNA and shRNA**

(a & b) Western blots showing the levels of phospho-TBK1, TBK1 and after transfection with two different siRNA sequence targeting TBK1 (a) siRNA from SantaCruz (b) siRNA from Ambion. (c & d), Western blots showing the depletion of TBK1 after transducing with shTBK1 lentiviral construct in H460 and PC9.



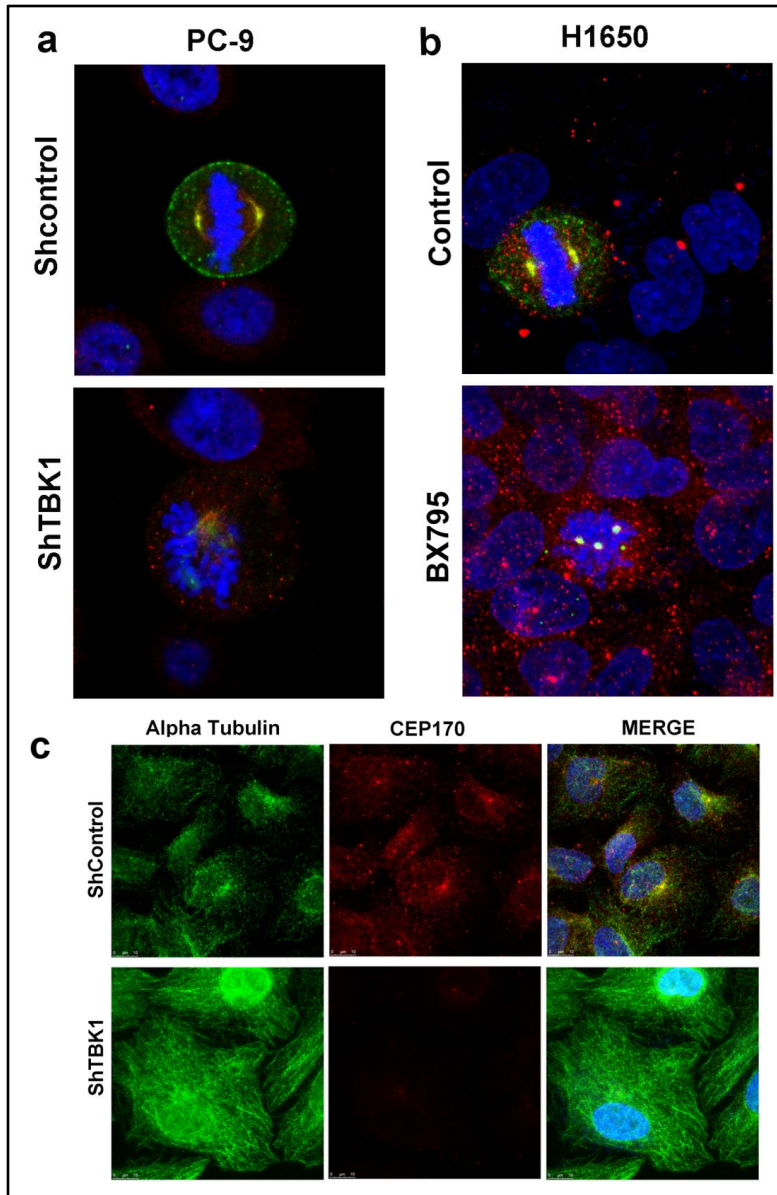
**Supplementary Figure 5. TBK1 inhibition increases number of centrosomes**

TBK1 inhibition in hydroxyurea (HU) treated U2OS cells using BX795 induced centrosome amplification. TBK1 inhibition alone was sufficient to induce centrosome amplification in 22% of cells; the possibility exists that this is due to incomplete cell division.



**Supplementary Figure 6. Colocalization of phospho-TBK1 with CEP170 in immune cells.**

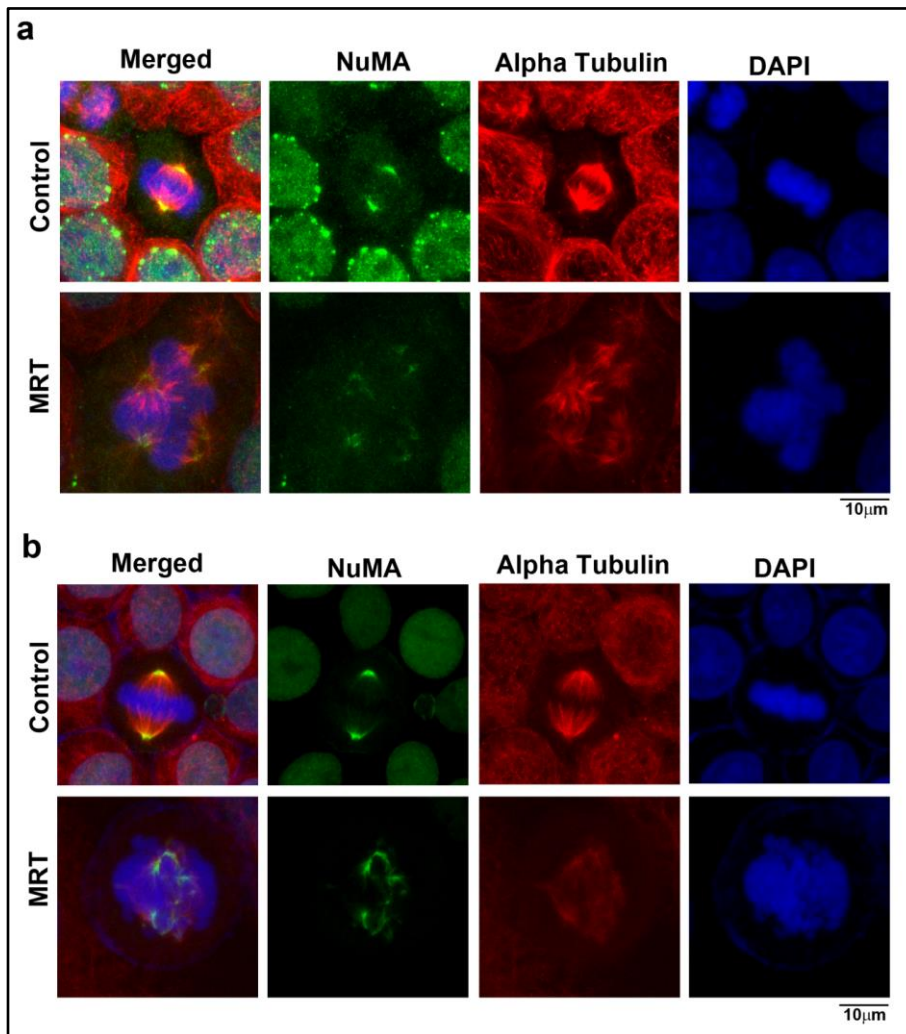
(a,b) Confocal image showing the co-localization of phospho-TBK1 (green) and CEP170 (red) in U937 (histiocytic lymphoma cells) and Daudi (Burkitt's lymphoma; B lymphoblast) cells. Scale bar=10  $\mu$ m.



**Supplementary Figure 7. Inhibition of TBK1 results in mitotic defects.**

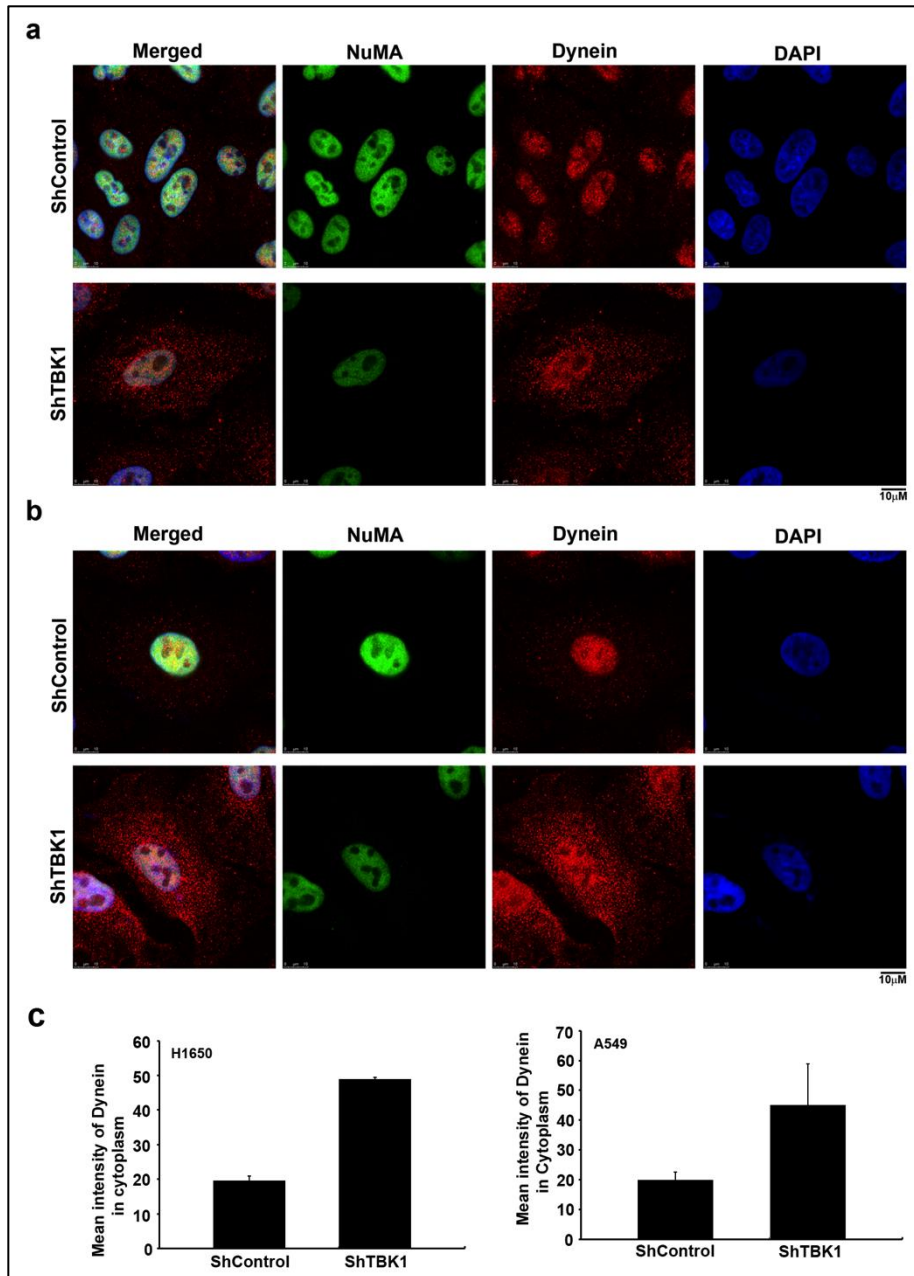
(a, b) Mitotic defects observed after depletion of TBK1 in PC9 (a) and inhibition of TBK1 by BX795 in H1650 (b). phospho-TBK1 (green), CEP170 (red) and DAPI (blue). Scale bar=10  $\mu$ m. (c) Depletion of TBK1 results in inhibition of CEP170 localization to the centrosomes in non-mitotic cells. Microtubules are visualized by staining for Alpha Tubulin (green), CEP170 (red) DNA (DAPI, Blue).





**Supplementary Figure 8. TBK1 inhibition affects spindle pole localization of NuMA.**

Inhibition of TBK1 by 2  $\mu$ M MRT affected spindle pole localization of NuMA. H460 (a) and HeLa (b) cells were stained for NuMA (green), Alpha Tubulin (red), DAPI (Blue). Scale bar 10  $\mu$ m.

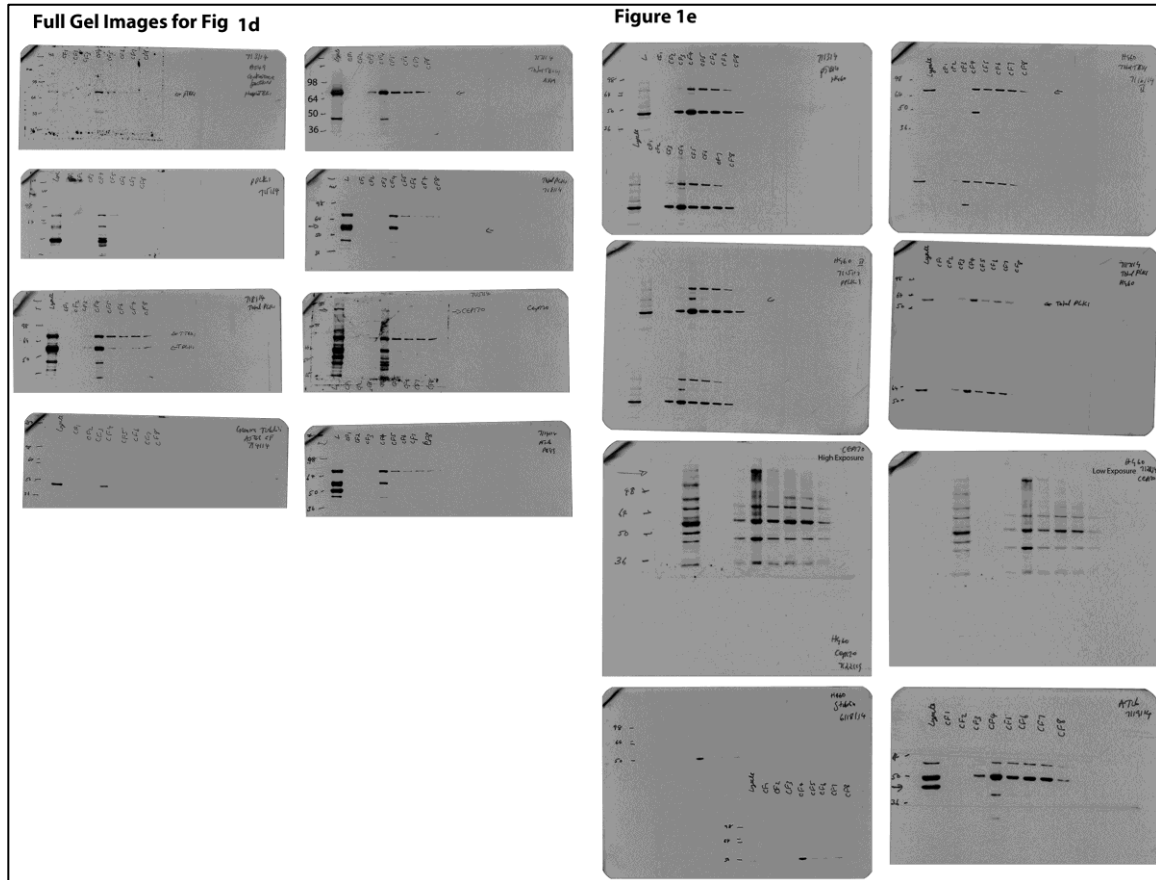


**Supplementary Figure 9. TBK1 depletion affects dynein levels in cytoplasm.**

Immunofluorescence staining for Dynein intermediate chain (red) and NuMA (green) in shcontrol and shTBK1 transfected H1650 (a) and A549 (b) cells. Scale bar 10  $\mu$ m. (c) Quantification of signal from immunofluorescence images showing alteration in Dynein intermediate chain levels in the cytoplasm after depletion of TBK1.

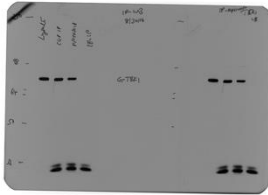
## Supplementary Figure 10. Uncropped images of western blots and gels.

The full scans of the important western blots and gels used in Figures 1, 2, 5, 6, 7 and 8

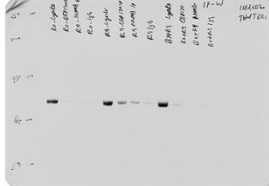




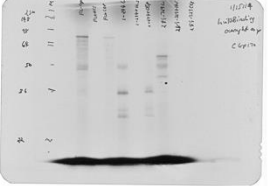
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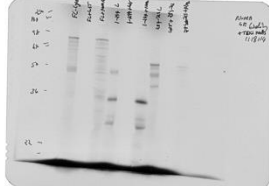
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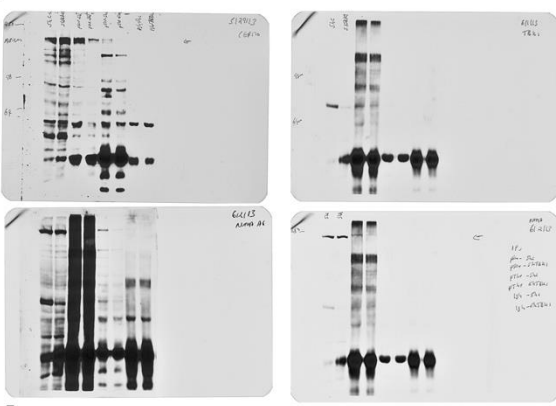
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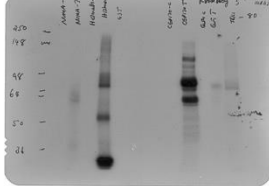
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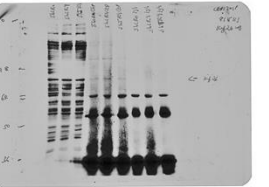
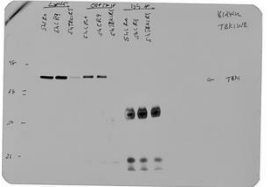
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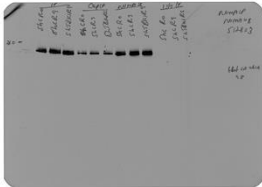
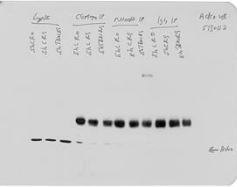
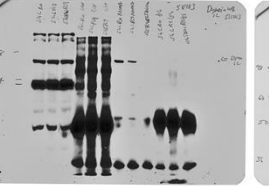
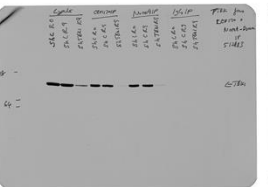
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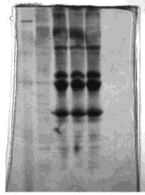
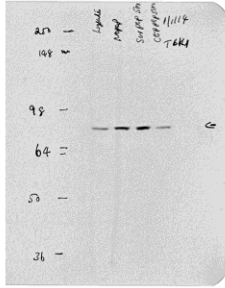
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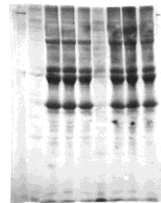
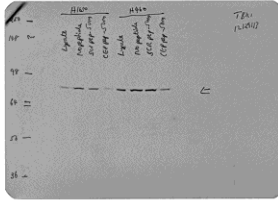
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7a

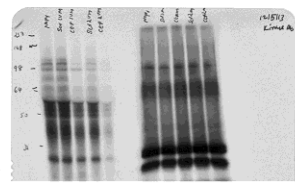


Coomassie stain

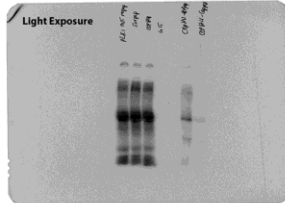
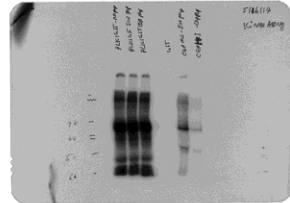


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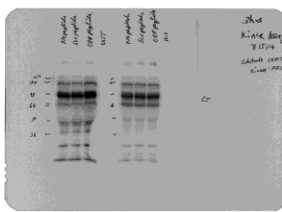
7b



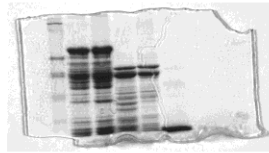
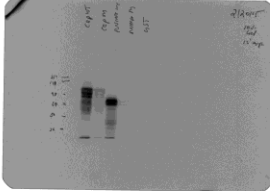
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7d

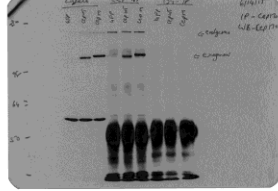
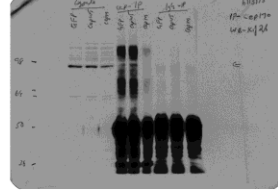


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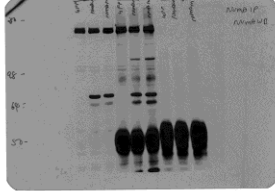
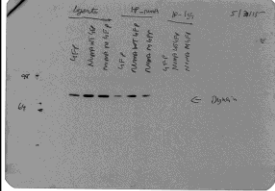


Coomassie stain

8d



8e



**Supplementary Table 1**

Peptide	Probability (%)	Start-Stop
QTSSTP <b>SS</b> LALTSASR	95	859-874
ERSE <b>S</b> LDPDSSMDT <b>T</b> LILK	95	877-895
DNS <b>S</b> PE <b>S</b> DVDTA <b>S</b> T <b>S</b> LVTGETER	95	926-950
LGEASDSELADADKA <b>S</b> VASEVSTTS	95	1108-1141
LQSAGSAMPT <b>SS</b> SFK	95	1268-1282
VFDE <b>S</b> LNFR	95	1358-1366
ALHPAAVSAAAEFENA <b>S</b> EAD <b>F</b> SIHFNR	95	1543-1570

**Phosphorylated residues on CEP170 identified by mass spectrometry.**

Phosphorylated Serine is indicated as '**S**'  
Phosphorylated Threonine is indicated as '**T**'

For identifying CEP170 amino acid residues phosphorylated by TBK1, *in vitro* kinase assays were performed with purified His tagged CEP170 in the presence of TBK1. *In vitro* kinase reaction without TBK1 was performed as the negative control and the residues phosphorylated specifically in the presence of TBK1 were identified by mass spectrometry.

**Supplementary Table 2**

<b>Peptide</b>	<b>Probability (%)</b>	<b>Start-Stop</b>
EPQAKPQLDLSIDSLDL <b>S</b> CEEGTPLSITSK	100	1711-1740
LPPKVESLE <b>S</b> LYFTPIPAR	100	1763-1781
LGSPDYGNSALL <b>S</b> LPGYRPTR	95	1860-1881
R <b>S</b> MQPIQIAEGTGITTR	100	1967-1984
KLG <b>S</b> LLR	99	2058-2065

**Phosphorylated residues on NuMA identified by mass spectrometry**

For identifying NuMA amino acid residues phosphorylated by TBK1, *in vitro* kinase assays were performed with purified GST tagged NuMA in the presence of TBK1. *In vitro* kinase reaction without TBK1 was performed as the negative control and the residues phosphorylated specifically in the presence of TBK1 were identified by mass spectrometry. Phosphorylated Serine is indicated as '**S**'