Supplementary Material and Methods

1- *TUNEL Assay for HeLa Xenografts and Immunocytochemistry* –TUNEL staining for both xenograft tumors and cultured cells was performed using ApopTag® Red *In Situ* Apoptosis Detection Kit, according to manufacturer's instructions (Millipore, catalog no. S7165).

2- *Xenograft* – Athymic NCr- nu/nu female mice (NCI, catalog no. 01B74) were split into two groups of 6 mice each. Each mouse was injected subcutaneously with 1 x 10^6 HeLa cells harboring doxycycline-inducible UNC45A shRNA or scramble shRNA. Mouse tumors were measured weekly. Daily intraperitoneal injections of doxycycline were initiated when tumors measured 100 mm^3. Treatment lasted for 6 weeks.

3- *Cell Proliferation Assay* - HeLa cells were grown on 24-well plates. UNC45A knockdown was achieved by transient transfection with UNC45A siRNA or exposure to 1 μg/mL doxycycline. Cells were analyzed at 24, 48, 72, and 96 hours post-transfection using CellTiter 96® AQ_{ueous} One Solution Reagent (Promega, catalog no. G3580). Plates were read with a Safire plate reader (Tecan, Australia).

4- *Centrosome Separation and Purification* – Centrosome separation and purification were achieved following the Cold Spring Harbor protocol, *Isolation of*

Centrosomes from Cultured Mammalian Cells, by Thomas E. Meigs and Daniel D. Kaplan (*Cold Spring Harb Protoc;* doi: 10.1101/pdb.prot5039).

Supplementary figure legends

Figure S1. (A) UNC45A is essential for the proliferation of breast and prostate cancer cells. Breast cancer cell lines HMLER and MDA-MB-453 and prostate cancer line LNCaP. Cells were transfected with 75 nM siRNA control (blue) or siRNA to UNC45A (red) and analyzed for proliferation using the. MTT assay. (B) Silencing UNC45A induces apoptosis in HeLa cells *in vitro*. Light microscope images showing HeLa cells with membrane blebbing in UNC45A knockdown cells 48h after transfection with 100 nM siRNA. (C and D) TUNEL analysis of HeLa cells in culture confirming the ongoing apoptosis. Average of apoptotic cells found in four microscopic fields comparing the TUNEL positivity in control and siRNA treated cells.

Figure S2. The polyclonal UNC45A antibody is specific to UNC45A. HeLa cells transfected with control or UNC45A siRNAs were immunoblotted with the poly clonal antibody raised against the extreme C-terminus of UNC45A..

Figure S3. Immunocytochemistry of asynchronous HeLa cells stained with DAPI (blue) and the polyclonal antibody specific to the C-terminus of UNC45A(red). Centrosomes were stained with antibody to the centrosomal protein γ-tubulin (green).

Figure S4. Myosin II does not localize to centrosomes. HeLa cells were stained with polyclonal antibody specific to myosin II (red). Centrosomes are visualized with the centrosomal protein γ-tubulin (green).

Figure S5. Immunocytochemistry of asynchronous U2OS cells stained with DAPI and the polyclonal antibody specific to the C-terminus of UNC45A (red) or phosphorylated Chk1 at serine 345 (pChK1 S345) (red). Centrosomes are visualized by staining for the centrosomal protein γ-tubulin (green).

Figure S6. Immunocytochemistry of asynchronous HeLa cells stained with DAPI and the polyclonal antibodies specific for Chk1 (red) or phosphorylated Chk1 at serine 345 (pChK1 S345) (red). Centrosomes are visualized with the centrosomal protein γ-tubulin (green).

Figure S7. Immunocytochemistry of asynchronous HeLa cells stained with DAPI and the monoclonal antibody against amino acids 1-476 of ChK1 (Santa Cruz, catalog no. sc-8408) (green). Centrosomes are visualized with the centrosomal protein pericentrin (red). DAPI images are not shown in the top panels.

Figure S1

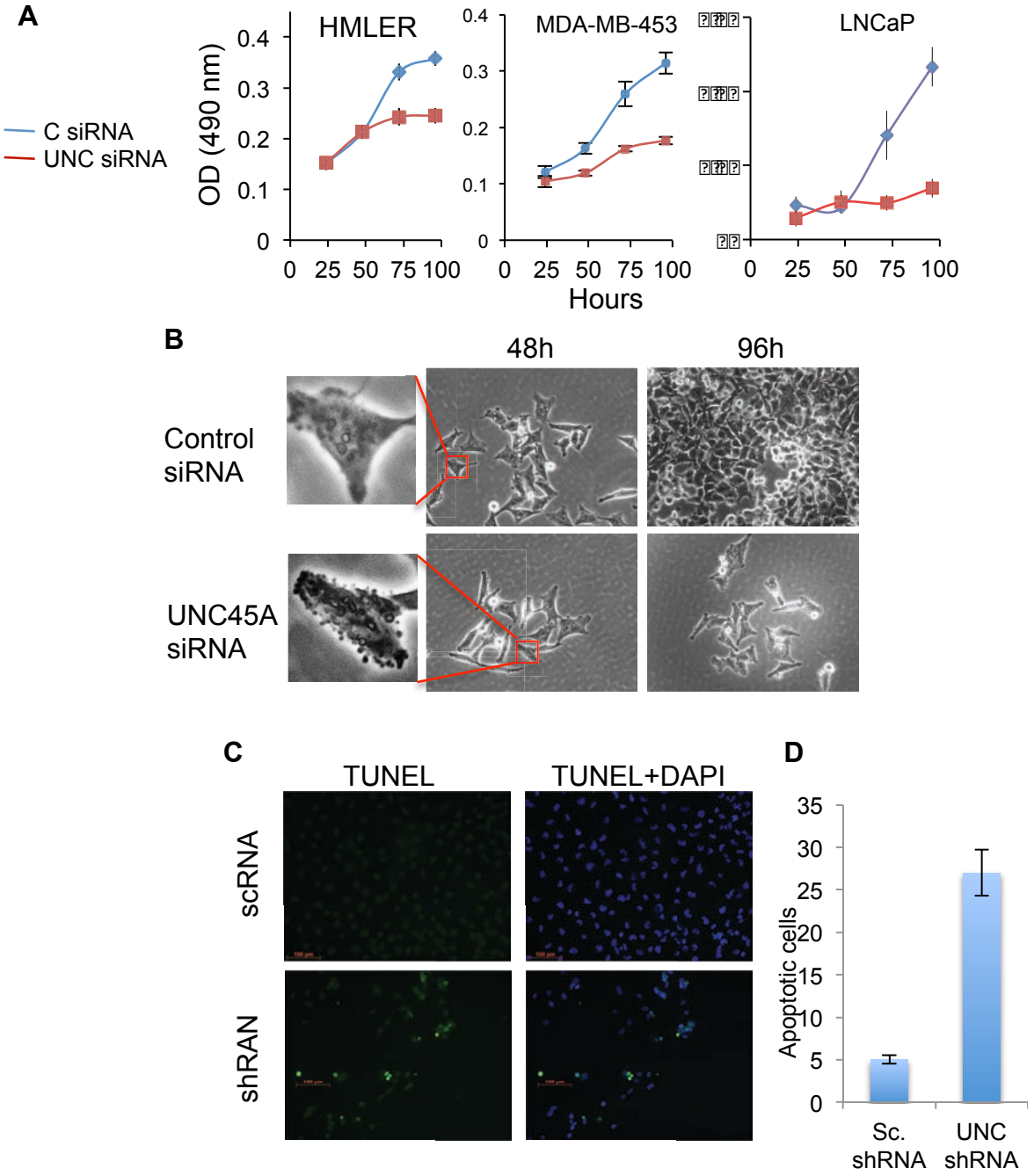
A
C siRNA
UNC siRNA
OD (490 nm)
HMLER
MDA-MB-453
LNCaP
Hours
B
48h
96h
Control siRNA
UNC45A siRNA
C
TUNEL
TUNEL+DAPI
scRNA
shRAN
D
Apoptotic cells
Sc. shRNA
UNC shRNA

Figure S2

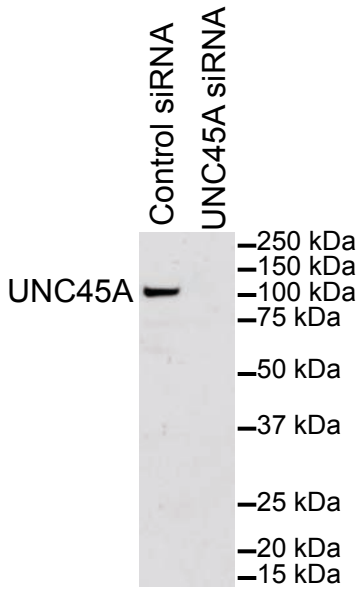
Control siRNA
UNC45A siRNA
UNC45A
250 kDa
150 kDa
100 kDa
75 kDa
50 kDa
37 kDa
25 kDa
20 kDa
15 kDa

Figure S3

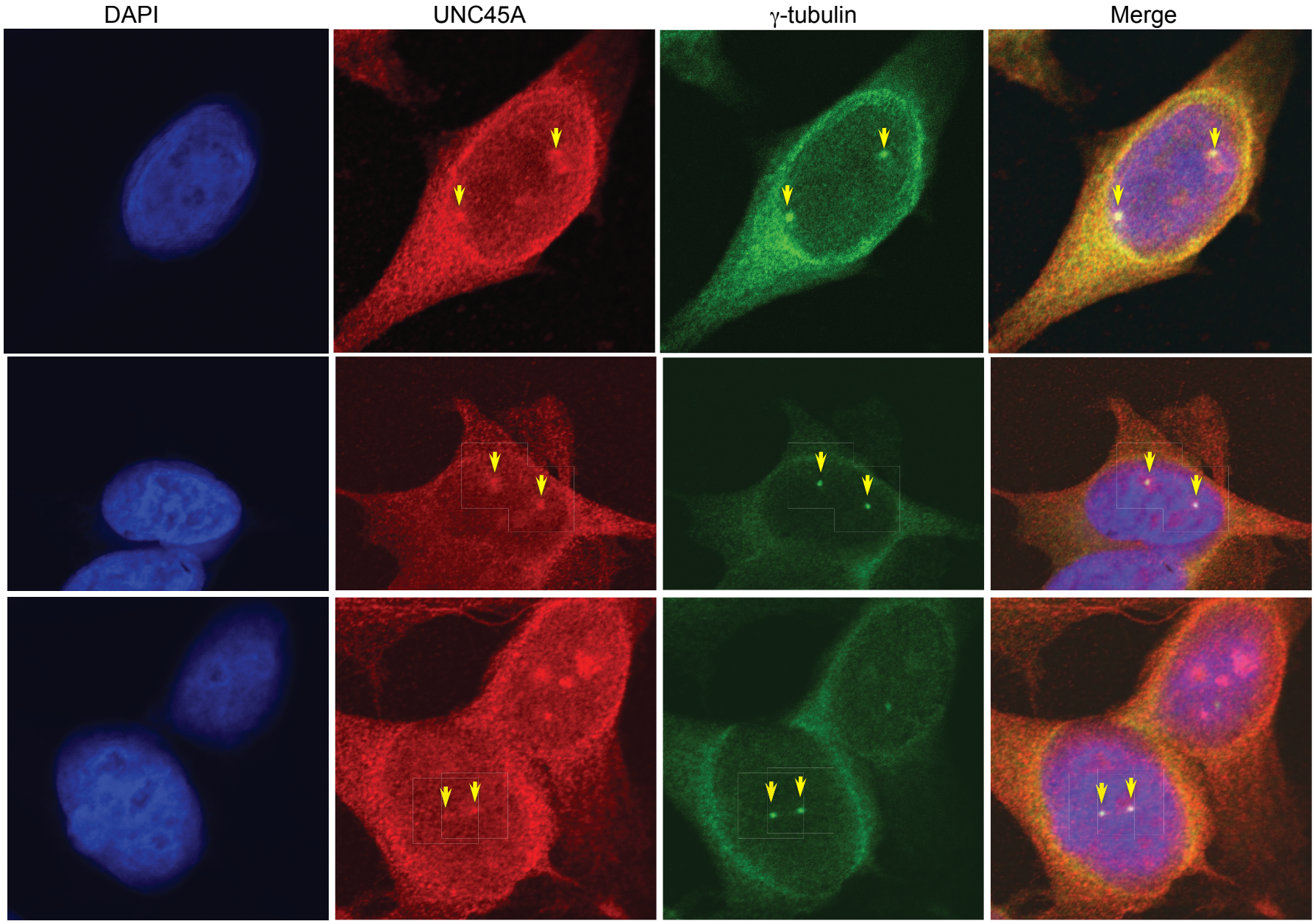

Figure S4

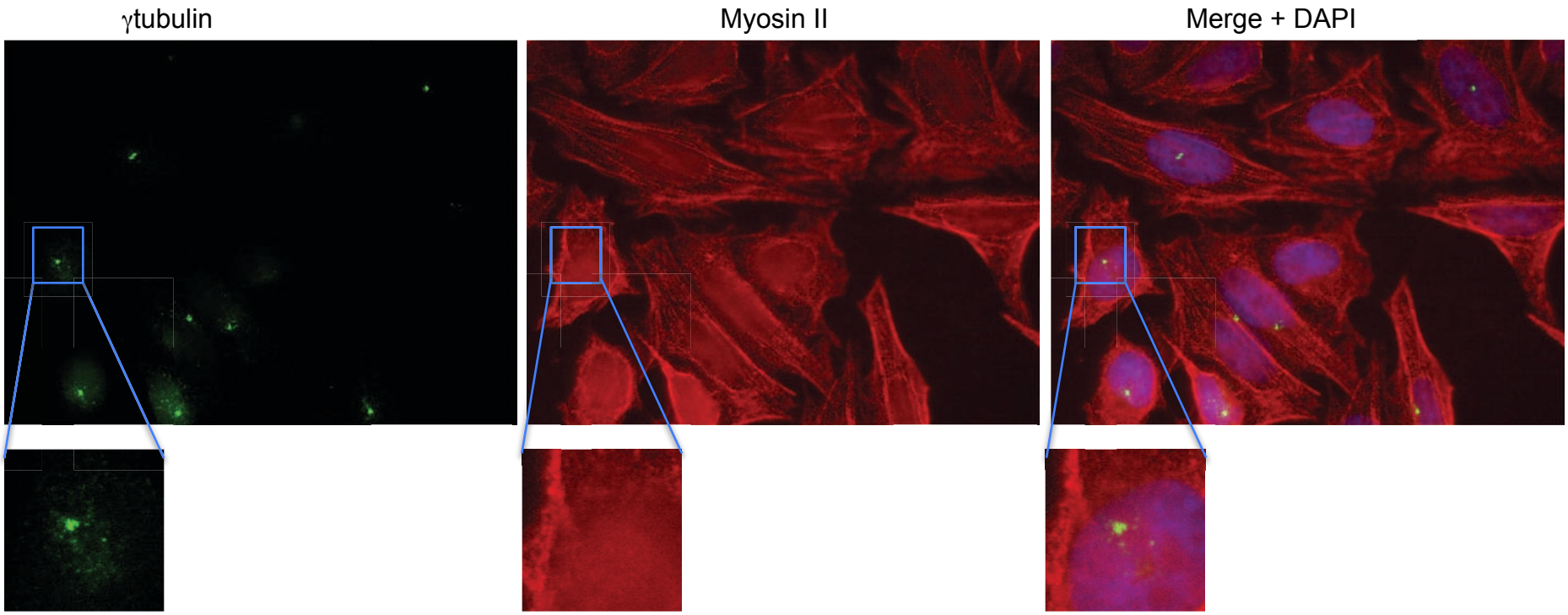

Figure S5

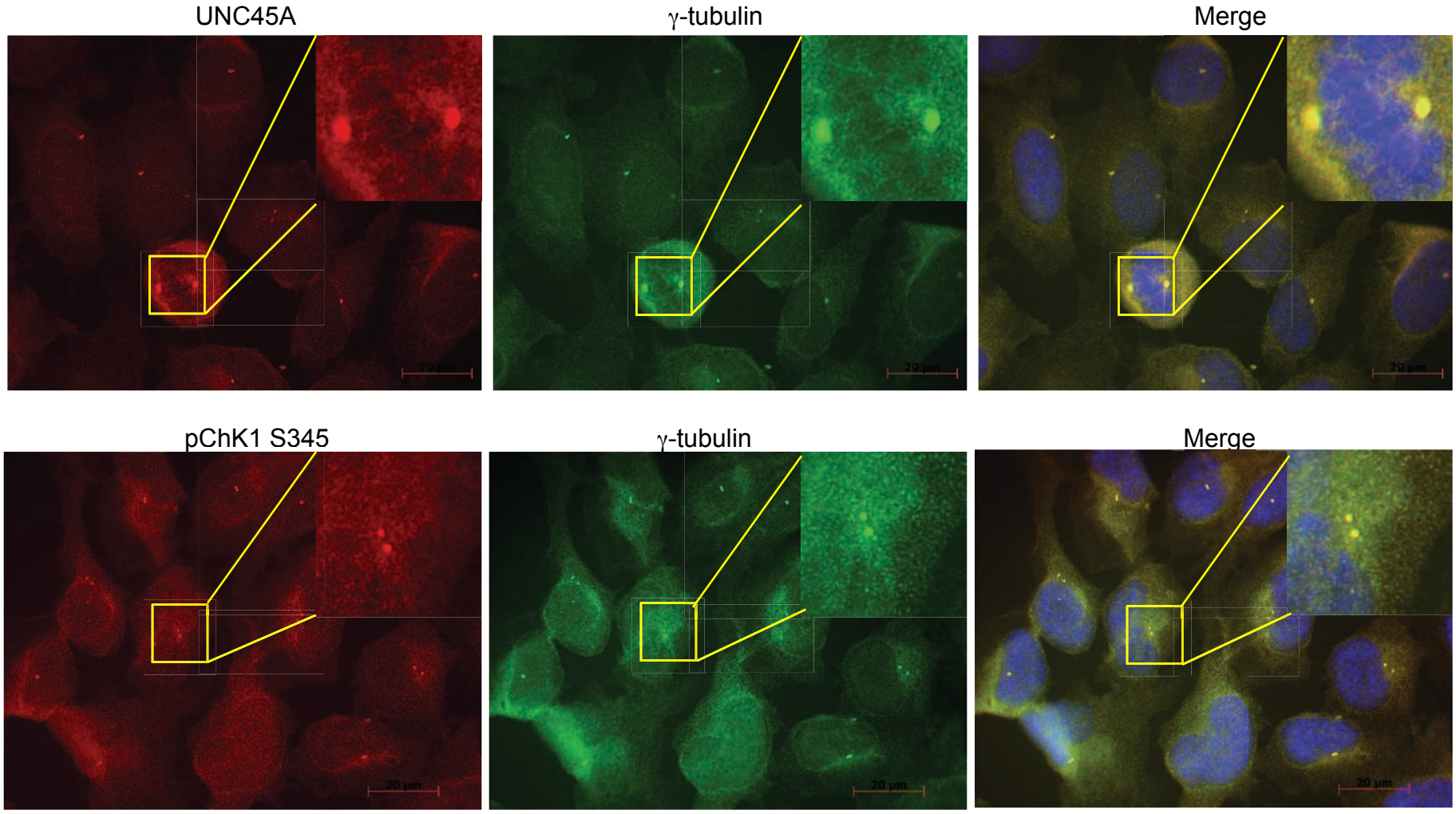

Figure S6

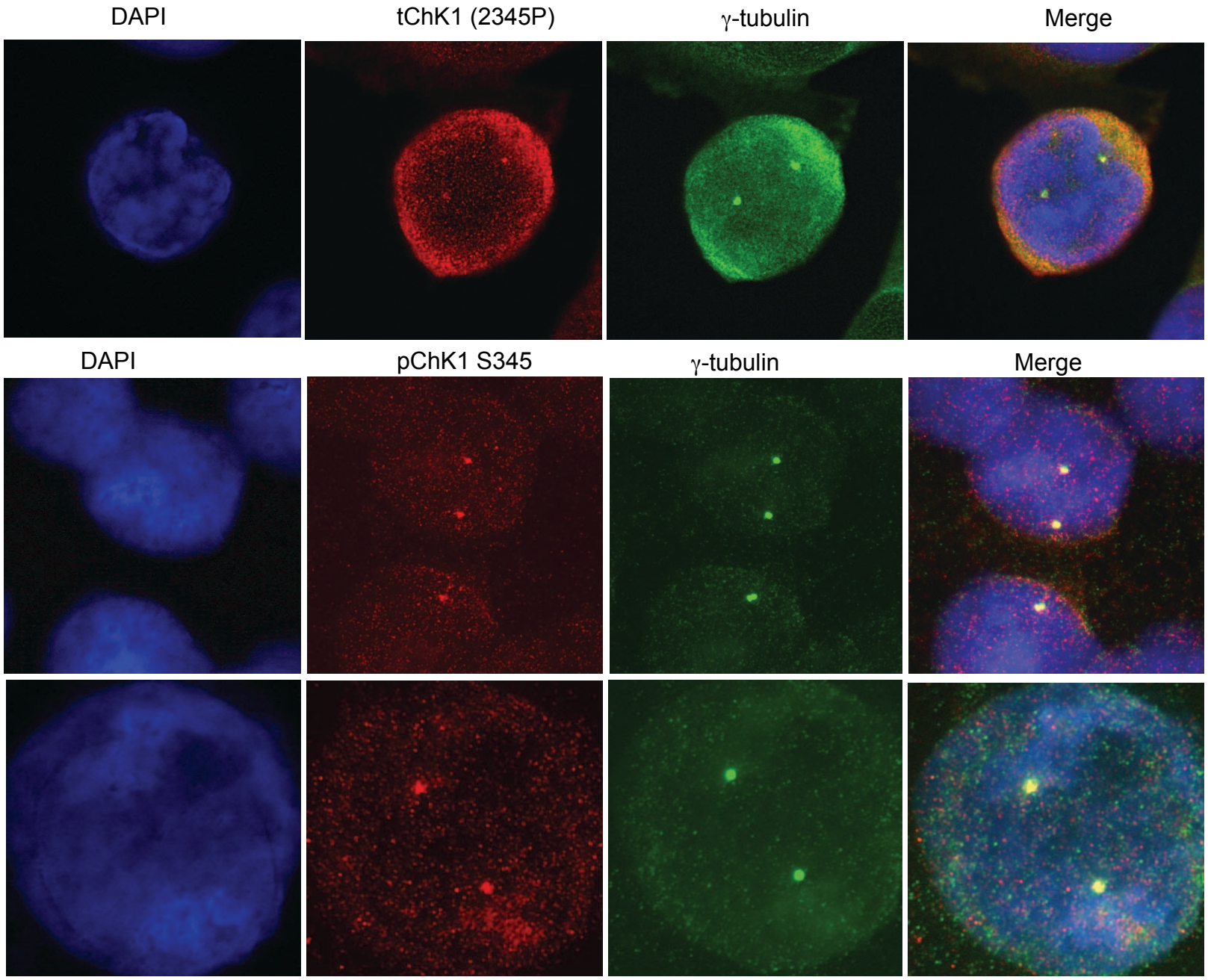
DAPI
tChK1 (2345P)
γ-tubulin
Merge
DAPI
pChK1 S345
γ-tubulin
Merge

Figure 7