Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Magee-Womens Research Institute and Foundation

2. **Reporting Period (start and end date of grant award period):** 01/01/2011-12/31/2011

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Cheryl A. Richards, MBA

4. **Grant Contact Person's Telephone Number:** (412) 641-8932

5. **Grant SAP Number:** 4100054859

6. **Project Number and Title of Research Project:** 4 - *Microtubule Post-Translational Modifications and Centrosome Dynamics During Mitosis in Normal and Cancerous Cells*

7. **Start and End Date of Research Project:** 01/01/2011- 12/31/2011

8. **Name of Principal Investigator for the Research Project:** Calvin R. Simerly, Ph.D.

9. **Research Project Expenses**.

   9(A) Please provide the amount of health research grant funds spent on this project for the entire duration of the grant, including any interest earned that was spent:

   $208,290

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
C. Simerly	Principal Investigator	45%	$75,533.44
G. Schatten	Co-Investigator	10%	$28,903.43
C. Redinger Hartnett	Senior Research Associate	10%	$7,566.89

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
None		

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____No_X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes___X_____No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐ NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)			

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____X___No_____

If yes, please describe your plans:

We are seeking NIH Research sponsorship for our findings that molecular motors and centrosome organizers are unique targets for cancer therapeutics.

12. Future of Research Project. What are the future plans for this research project?

This research is generating the foundation for studies seeking to develop more specifically focused chemotherapeutic agents. We are interested in discovering whether the molecular motors and organization of the mitotic spindles in cancer cells have specific differences from somatic cells, so that the next generation of chemotherapeutic agents might be constructed to target the cancer cells and not the molecular motors found in brain or other non-cancerous cells.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____No_____X_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____No____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____X_____No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

This sponsorship was invaluable for the investigators to develop new sophisticated dynamic approaches for investigating microtubule-mediated motility in living cancer cells using FRAP, and has been instrumental in recruiting new National Cancer Institute resources to programs here. Furthermore, this sponsorship has been leveraged to recruit new collaborations to the Commonwealth and we are optimistic that further dividends will accrue.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of

your institution (e.g., entire university, entire hospital system)?

Yes_____No_____X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____No_____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____No_____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written

response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Goals and Objectives: Microtubule defects and centrosome aberrations cause cancers and birth defects, since they induce chromosome aneuploidies after mitosis in both somatic and embryonic cells. They are responsible for inherited disorders and their functioning is essential for brain activities. Consequently, their activities span life from conception through death. While cell biologists a century ago were as familiar with the centrosome ('the cell's central body') as they were with chromosomes ('the cell's colored bodies' because they bind cytological stains), progress in characterizing the molecular constituents and mechanisms responsible for functional activities has paled when comparing centrosome molecular biology with chromosome molecular biology. Nevertheless, the essential roles of the centrosome for normal cell function are now incontrovertible and a panoply of diseases and disorders results from microtubule and centrosome dysfunctions or 'centrosomopathies.' Here we will characterize the vital permanent molecules in the mitotic centrosome and discover which reside temporarily at the centrosome, along with the post-translational modifications of microtubules which occurs during normal and cancerous cell cycles.

Specific Aims: Significant progress was accomplished in all four specific aims and did not change over the funded period of this application. The specific aims include:

1. Do cancer cells display differences in the delta-2 post-translational modification of alpha-tubulin as compared with noncancerous cells?
2. Using dynamic confocal imaging with a living marker for centrioles, does GFP-centrin behave aberrantly in cancer cells as compared with controls?
3. Do the molecules which reside at the centrosome differ between cancer cells and controls?
4. Do the anticancer drugs currently used target these posttranslational modifications of alpha tubule, centrioles and/or these centrosomal molecular and can new drugs be discovered.

Project Summary.

Aim 1. Do cancer cells display differences in the delta-2 post-translational modification of alpha-tubulin as compared with noncancerous cells?

We tested an affinity purified antibodies to delta-2 (Δ-2) tubulin and acetylated α-tubulin in non-cancerous lung fibroblast cell line (WI-38), a lung carcinoma line (NCIH292) and a breast cancer cell line (MCF7), all purchased from ATCC (Manassas, VA). WI-38 lung fibroblast were grown in Minimal Essential Media (MEM) + 10% fetal bovine serum (FBS) , NCI-H292 lung carcinoma line was grown in RPMI media (ATCC) with 10% FBS and MCF7 breast cancer

epithelial line was grown in MEM + 10% FBS with 0.01 mg/ml human insulin (Sigma, St. Louis, MO) on sterile 22mm^2 glass coverslips. After a brief rinse in warm sterile PBS, fixation was accomplished by plunging coverslips into cold (-20ºC) absolute methanol for 8-10 minutes followed by rehydration in PBS+ 0.5% Triton X-100 detergent (PBS-TX). After blocking for 1 hr in PBS-TX containing 10% normal goat serum, 3 mg/ml BSA, and 150 mg/ml glycine to reduce non-specific antibody binding (PBS blocking solution), fixed cell lines were incubated overnight in a cocktail of primary antibodies including Δ-2 rabbit anti-tubulin antibody (1:200), YOL134 anti-rat total tubulin antibody (1:200) and mouse 611B-1 acetylated α-tubulin (1:100) at 4ºC. Following a 30 min rinse in PBS-TX, primary antibodies were detected with a 1:100 dilution of fluorescein-conjugated goat anti-rabbit IgG, rhodamine conjugated goat anti-rat IgG and Cy5 conjugated anti-mouse IgG secondary antibodies applied overnight at 4ºC. After a penultimate rinse in PBS-TX, stained cells were mounted in antifade solution (Vectashield, Vector Labs, and Burlingame, CA) with DAPI for detecting DNA. All slides were examined with an A1 laser-scanning confocal microscope with a x100 Plan Fluor objective (NA=1.3) and the detection of Δ-2 tubulin, total microtubules and acetylated tubulin recorded for mitotic and interphase cells in all 3 cell lines. Control slides were processed as above but without primary antibodies, and no nonspecific staining of microtubules or centrioles was observed.

α-Tubulin within older stable microtubules (MTs) often undergo posttranslational modifications (PTMs), including acetylation at lysine 40, loss of the carboxyl-terminal tyrosine (termed detyrosination), and further loss at this terminus (called Δ-2 tubulin). New theories have recently emerged regarding the Tubulin Code, suggesting that cytoplasmic PMTs of MTs may control cellular fates, including in cancer cells. Centrosomes, composed of a pair of centrioles and the pericentriolar material (PCM), are vital to assembly of interphase microtubules and the mitotic spindles during cell division. Furthermore, the connection between centrosome abnormalities and cancer, first proposed in the early 19th century by T. Boveri, has been intensely revisited to explore whether new avenues exist for the diagnosis, prognostic and therapeutic approaches in cancer. Our analysis of fixed and stained slides suggest that Δ-2 tubulin is a general constituent of all centrioles, being expressed at mitotic and interphase centrioles in noncancerous and cancer cell alike but absent in spindle or cytoplasmic microtubules (Figure 1). We found no significant differences in expression of Δ-2 tubulin after counting over 50 mitotic and interphase cells. Acetylated α-tubulin, another form of PTM of MTs, was also detected in interphase and mitotic centrioles of all cell lines tested (Figure 2) with no significant differences detected between the lines. In mitotic spindles, acetylated α-tubulin was often observed weakly at the spindle poles in addition to the centrioles at each pole (metaphase centriole detection: WI 38: 93%; NCI H292: 100%; MCF7: 100%; n=14). At interphase, acetylated α-tubulin stained a subset of cytoplasmic microtubules in all 3 cell lines tested as well as centrioles adjacent to the cells nucleus (interphase centriole detection: WI 38: 73%; NCI H292: 90%; MCF7: 87%; n= 53).

Collectively, the data suggest that Δ-2 tubulin and acetylated α-tubulin do not vary between cancerous and noncancerous cell lines, although it is a highly useful marker for centriole detection. This suggests that centriole modifications and perhaps stability do not differ between cancer and noncancerous cells, a hypothesis we will test in the next Aim.

Aim 2. Using dynamic confocal imaging with a living marker for centrioles, does GFP-centrin behave aberrantly in cancer cells as compared with controls?

We successfully tested our GFP-centrin (pEGFP-CETN2) construct (donated generously by Dr. Jeffrey Salisbury, Mayo Clinic, MN) in WI 38 control lung fibroblast line as well as both NCI H292 and MCF7 cancer cell lines following the production of lentiviral particles using ViraPower™ Lentiviral Packaging Mix (Life Technologies, Carlsbad, CA). All cell lines were grown in T-25 cell culture flasks. When cells were 30-40% confluent a solution of 60µls of concentrated centrin-GFP lentivirus and 6mg/mL of polybrene (Sigma) in complete culture media was added to the cells and incubated overnight at 37°C. Expression of GFP centrin was determined to be > 75% of the cells as detected by inverted fluorescent microscopy. All lines were determined to be stably transduced based on GFP centrin detection following numerous cell passages as well as following cryopreservation and thawing for reconstituting cell lines in vitro (Fig. 3). We were able to detect cells with both normal centriole numbers (Fig. 3A-C: double arrows) as well as cells with over-duplicated centrioles (Fig. 3A-C: quad arrows) and at different stages of the cell cycle, including mitosis.

To explore centrosome behaviors between cancerous and non-cancerous lines, we plated each cell line independently on 35 mm sterile iBidi dishes (Research Products International, Mt Prospect, IL) coated with 0.1% gelatin and permitted attachment and growth for 24-48 hrs at 37°C in a 5% CO_2 incubator. Time-lapse video microscopy (TLVM; n= 9) was next performed on a T_i 90 Nikon inverted microscope equipped with Perfect Focus to prevent Z-axis drifting during prolonged live- cell imaging. Exposure to fluorescent light during imaging was attenuated by using combined ND4 / ND8 filters to reduce photodamage. Image capture every 15 minutes was accomplished using a Plan Fluor oil x100 objective (NA=1.3) using an Andor high resolution camera and Nikon Elements software for archival of data. Cells were placed in a Tokai stage incubator to maintain temperature at 37ºC and gas at 5% CO_2 throughout live cell imaging. Representatives of 9 TLVM trials are shown in Figure 4 and 5. Our experiments permitted us to image Centrin-GFP in cancer and non-cancer cells for extended times (up to 24 hrs) to observe centrin-GFP localization to centrosomes and how they functioned during a cell cycle, including mitosis and cell division. Figure 4 represents normal mitosis in a MCF7 cancer cell, showing GFP-centrin expression at single centrioles localized to each metaphase pole (Fig. 4A, arrowheads; double arrows: chromosome position). Subsequent frames over the next ~1.5 hr show this cell undergoing chromosome separation (Fig. 4B-F, arrows) and cell division (Fig. 4F, * denotes cleavage furrow). In Figure 5, we followed the fate of an interphase stage MCF7 cell with over-duplicated centrioles (Fig. 5A: arrowheads, pair of centrioles) during cell cycle progression. We observed all four centrioles first congressing within the cytoplasm (Fig. 5B: arrowheads) by 2 hrs after the start of TLVM imaging, followed by a rapid separation of centriole pairs (Fig. 5C-E: arrowheads) as this cell entered into mitosis within the next ~60 minutes (Fig. 5F). However, this cell failed to undergo cytokinesis after 3.5 hours of further imaging and despite using attenuated light exposure (Fig. 5G-H). Eventually, this cell re-entered interphase and the centrioles became more closely aligned. We concluded that GFP-centrin was expressed properly at the centrioles and that labeled cells could be followed dynamically for extended imaging periods to gain insight into centriole behavior during cell cycle progression.

To explore centrosome dynamics between cancerous and non-cancerous lines, we next

performed Fluorescence Recovery After Photobleaching (FRAP) experiments using centrin-GFP expressing cells under live-cell laser confocal microscopy using a 4 laser point scanning Nikon A1 confocal microscope and Elements software. We typically used the UV (405 nm) laser line to photoablate single or pairs of centrioles at both interphase and mitosis, monitoring recovery of fluorescence (i.e., the migration of new centrin-GFP into the centrioles) over 5-10 minutes. Laser ablation was performed using 15% laser power for 10secs at the marked region of interest (ROI). Fluorescent recovery was monitored for 5-10 minutes, acquiring images every 30 sec. Although the live cell DNA dye Hoechst 33342 was sometimes used to verify chromatin status, this compound was toxic to cell cycle progression after laser photoablation and thus we did not routinely employ this dye after initial experiments. For GFP-centrin labeled cells, we performed 11 Frap experiments with WI38 cell line (all interphase), 15 FRAP experiments with NCIH292 (8 interphase;7 mitotic), and 17 FRAP experiments with MCF7 (10 interphase: 7 mitotic). We also performed another 27 FRAP experiments with mCherry Tubulin labeled MCF7 (5 interphase:15 mitotic) and NCIH292 (4 interphase: 3 mitotic) cancer cells (data not shown). Figure 6 summarizes our findings from FRAP analysis of GFP centrin expressing cell lines. WI-38 GFP centrin labeled centrioles ablated with the 405 laser line generally saw full fluorescence recovery at centrioles within 10 minutes (Fig. 6A-D; n=4/11). Conversely, centrioles tagged with GFP centrin in cancer cells showed remarkably different dynamics, with no cells showing full GFP centrin fluorescence recovery in the centrioles between 5-10 minutes post-photoablation (NCI H292: Fig. 6E-H; n= 0/8 and MCF7: (Fig. 6I-L; n=0/10). Similar findings were observed in mitotic GFP centrin-expressing centrioles ablated and monitored for fluorescent recovery in NCI H292 and MCF7 cells (data not shown). These observations suggest that centrosomes in cancer cells may be more unstable than noncancerous cells, with centrin localization easily altered during interphase or mitosis in the centriole lumen.

Aim 3. Do the molecules which reside at the centrosome differ between cancer cells and controls?

We have investigated by immunofluorescence (ICC) nearly 20 different centrosome and centriole-specific affinity purified antibodies in WI-38, NCI H292, and MCF7 cell lines. The results of our finding are summarized in Table 1. The overall goal of this aim was to detect constituent proteins that are critical in centrosome assembly and function and to identify any variations observed between noncancerous and cancerous cell lines. Adhering cells to coverslips were processed as described in Aim 1 and fixation accomplished using one of three methods: i. cold, absolute methanol for 8-10 mins; ii. 2% paraformaldehyde in warm PBS for 15 min; and iii. permeabilization in a glycerol-based extraction buffer (Buffer M) containing 8% methanol and 1% Triton X-100. Following fixation and rehydration, cells were blocked in PBS blocking solution for 1hr before applying primary antibodies overnight at 4°C. After removal of primary and rinsing cells for 15 min in PBS, secondary antibodies were applied for an additional overnight incubation at 4°C. The penultimate rinse in PBS was performed followed by labeling DNA with Hoechst 33342 dye for 5 min before mounting slides in antifade solution. The rationale for performing the Buffer M/methanol fixation protocol is to confirm and compare the patterns of centrosome/centriole markers with those observed after formaldehyde or absolute methanol fixation. Together, these techniques provided confidence that the tested antibodies reliably describe a molecular characterization of the microtubule organizing centers (MTOCs) and are not fixation or labeling artifacts. Controls for both false positive and false negative

results were also performed on all cell lines. Secondary antibodies were not found to cross-react with microtubules, centrioles, chromosomes or nuclear elements, and produced very low background signals. All fixed specimens were observed and photographed by confocal microscopy.

Pericentriolar material (PCM) components like γ-tubulin and pericentrin appear to be well conserved and were equally identified in both noncancerous and cancerous cell lines. Likewise, both Δ-2 tubulin and 20H5 centrin antibodies localize to the centrioles in all three cell lines, including in cancer lines with spindle abnormalities (tripolar, tetrapolar spindles) and atypical centriole numbers at mitotic spindle poles. Resident proteins involved in the initiation, elongation, and maturation of centriole assembly were also investigated by ICC. Proteins involved in the assembly of the centrioles like Cep135 and HsSAS6 were strongly expressed in interphase and mitotic centrioles in all 3 cell lines tested. Similarly, proteins with known roles in microtubule (MT) capping, centriole anchoring, and scaffolding proteins for PCM proteins (ninein, cenexin1 and Cep 192) were largely conserved in noncancer and cancerous cell lines, although Cep 192 appeared more weakly detected in MCF7 breast cancer cells. Interestingly, we also observed that CP110, a protein involved in centriole duplication, was much more robustly detected in cancerous cell lines as opposed to the WI 38 control cell line. (Fig.7). Likewise, c-Nap1, involved in cohesion of parental centrioles, was not detected in either cancer cell line compared to the WI 38 control cell line. These observations are consistent with supernumerary centriole assembly and misalignment of centrioles found in many cancers cells. Collectively, these early observations suggest that specific centrosome molecules can vary between noncancerous and cancerous cell lines and may provide interesting scientific avenues to investigate how defects arising in malignant cells contribute to mitotic errors and cancer onset.

Aim 4. Do the anticancer drugs currently used target these posttranslational modifications of alpha tubule, centrioles and/or these centrosomal molecular and can new drugs be discovered?

To evaluate the effects of common chemotherapeutics on tubulin modifications and centrosome composition, we tested well established chemotherapeutic drugs on our WI 38, NCI H292, and MCF7 cell lines, including cyclophosphamide (CTX), cisplatin, paclitaxel, vinblastine, and vincristine. All drugs were prepared as 10 mM stocks in DMSO and stored in frozen aliquots. Cell lines growing on coverslips were incubated in 1μM of each drug for 24 hrs prior to fixation in absolute cold methanol as described in Aim1. Control cells were incubated in DMSO solvent at an equal volume (1:10,000). Fixed cell were stained with pericentrin antibody 4448 (1:600), YOL 1/34 general anti-tubulin (1:200) and 611B-1 acetylated α-tubulin (1:100) and analyzed by confocal microscopy as described in Aim1. For each drug treatment, we observed and recorded the mitotic index, centrosome numbers and centrosome position (split, migrated apart versus adjacent, adhering) for a minimum of 100 cells. We observed a significant increase in mitotic arrest in all cell lines after treatment with paciltaxel (taxol), vinblastine, and vincristine drugs compared to control, cisplatin and CTX (Fig 8, top graphs). Atypical centrosome counts (1, 3, 4 or more) were significantly increased after paciltaxel, vinblastine and vincristine exposure in all 3 cell lines relative to control, cisplatin and CTX treatments (Fig 8, middle graphs). Finally, we observed centrosome segregation to be significantly impacted after paciltaxel or vinblastine exposure in all 3 cell lines investigated (Fig 8, bottom graphs). Curiously, vincristine did not appear to impact centrosome segregation in WI-38 cells but did show increased blocking of

centrosome splitting in HCI-H292 and MCF7 cancer cells. The mechanism behind this observation is not yet understood. Microtubules were completely absent in all cells treated with vinblastine and vincristine (Fig. 9A-C), but significantly enhanced after paciltaxel treatment (Fig.9D-F).Interestingly, we also observed a large increase in paracrystalline arrays in the cytoplasm of NCI-H292 lung carcinoma cells after 1µM vincristine treatment relative to WI-38 control lung fibroblast or MCF7 breast cancer cells (Fig 9A-C), perhaps indicating that NCI-H292 cells are more susceptible to vincristine *in vitro* than for control or other cancer cells.

Novel targets for cancer therapy involving inhibition of histone deacetylation (HDACs) have recently been discovered. A unique member of the HDAC family, HDAC6, has been shown to target α-tubulin as its major substrate as opposed to histones, and thus it has been categorized as a tubulin deacetylase (TDAC). Reports showing over-expression of HDAC6 in a diverse group of tumors and cancer cell lines suggests that this protein has an important role in many cancers and drugs that target HDAC6 are now being investigated as new methods for cancer therapy. We investigated a number of HDAC inhibitors (HDACi) on microtubule and centriole acetylation patterns, including Tubastatin A (TubA: 100nM-1µM), suberoylanilide hydroxamic acid (SAHA; 100nM-1µM), sodium butyrate (NaB; 100µM-1mM), and valproic acid (VPA; 500µM- 1mM). All cell lines were treated for 48hrs with various concentrations of HDACi's prior to fixation in absolute methanol for 10 min. After rinsing, fixed cells were blocked in PBS blocking solution and immunostained with antibodies to Δ-2 tubulin, YOL 1/34, and acetylated α-tubulin as describe in Aim 1. Analysis of all three cell lines revealed that HDACi did not affected Δ-2 tubulin expression at the centrioles (not shown) but did abolish centriole acetylated α-tubulin detection (Fig. 10D-O). Conversely, only the known HDAC6 inhibitors TubA (Fig. 10D-F) and SAHA (Fig 10G-I) increased spindle microtubule acetylation relative to untreated control cells. The non-HDAC6i VPA (Fig. 10J-L) and NaB (Fig. 10M-O) did not cause microtubule hyperacetylation. Collectively, these data suggest that inhibition of HDAC6 specifically affects centriole and microtubule acetylation patterns in cancer cells. Since microtubules and centrioles play significant roles in cell cycle divisions, HDAC6i may provide a novel mechanistic approach to understanding cancer onset at the cellular level.

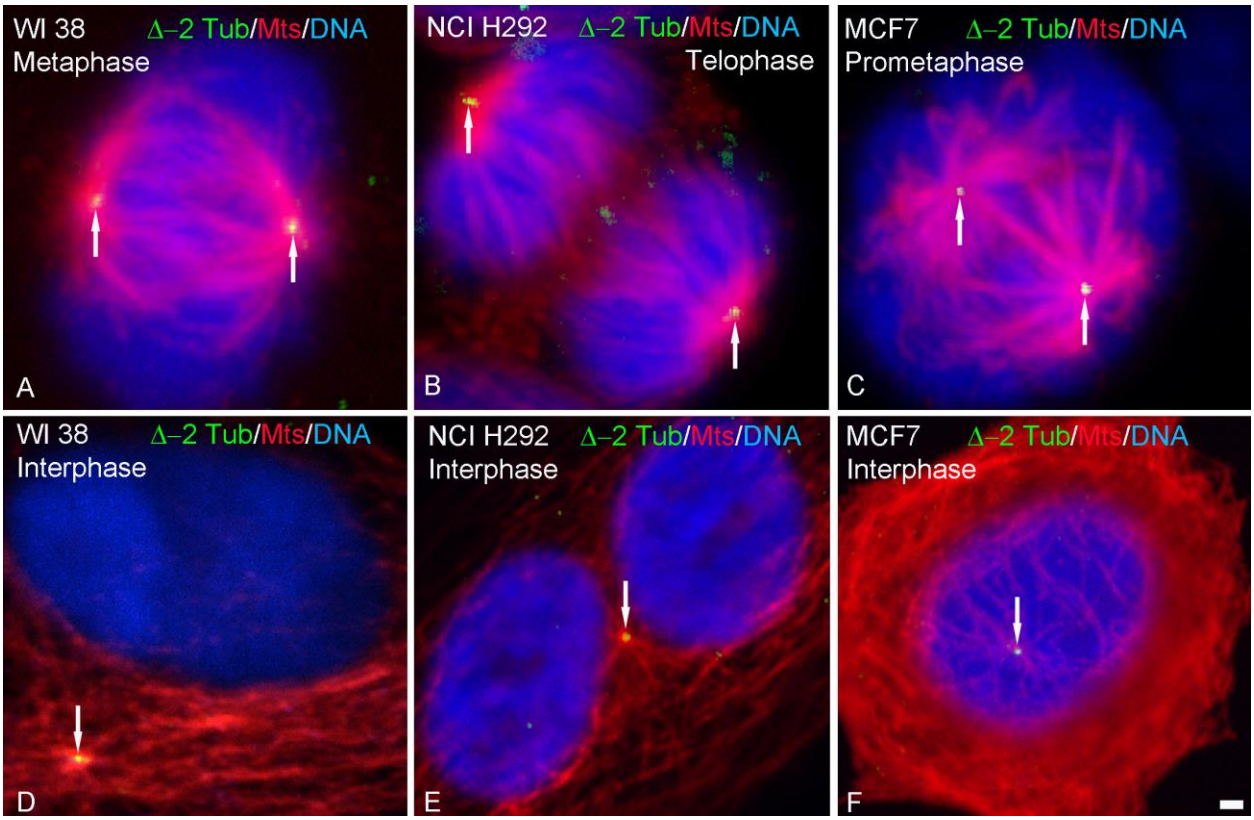

Figure 1. Detection of delta-2 (Δ-2) tubulin in control WI 38 and the cancer cell lines NCI H292 and MCF. **A and D**: Δ-2 tubulin localizes to mitotic metaphase (A: green, arrows) and interphase (D: green, arrow) centrioles, but not the mitotic spindle or cytoplasmic interphase microtubule arrays (A and D: red, microtubules; blue, DNA). **B and E**: Δ-2 tubulin detection in NCI H292 lung carcinoma cell at anaphase. Both mother and daughter centrioles at the spindle poles are labeled with Δ-2 tubulin (B: green, arrows). In an abnormal NCI H292 cancer cell at interphase depicting a duplicated nucleus (E: blue, DNA), Δ-2 tubulin is observed at the single centriole (E: green, arrow) lying within the microtubule aster (E: red, microtubules). **C and F**: Δ-2 tubulin localization to prometaphase centrioles (C: green, arrows) and the interphase centriole (F: green, arrow) within the microtubule astral array (F: red, microtubules; blue, DNA). All images triple-labeled for Δ-2 tubulin (green), microtubules (red) and DNA (blue). Scale bars= 1µm.

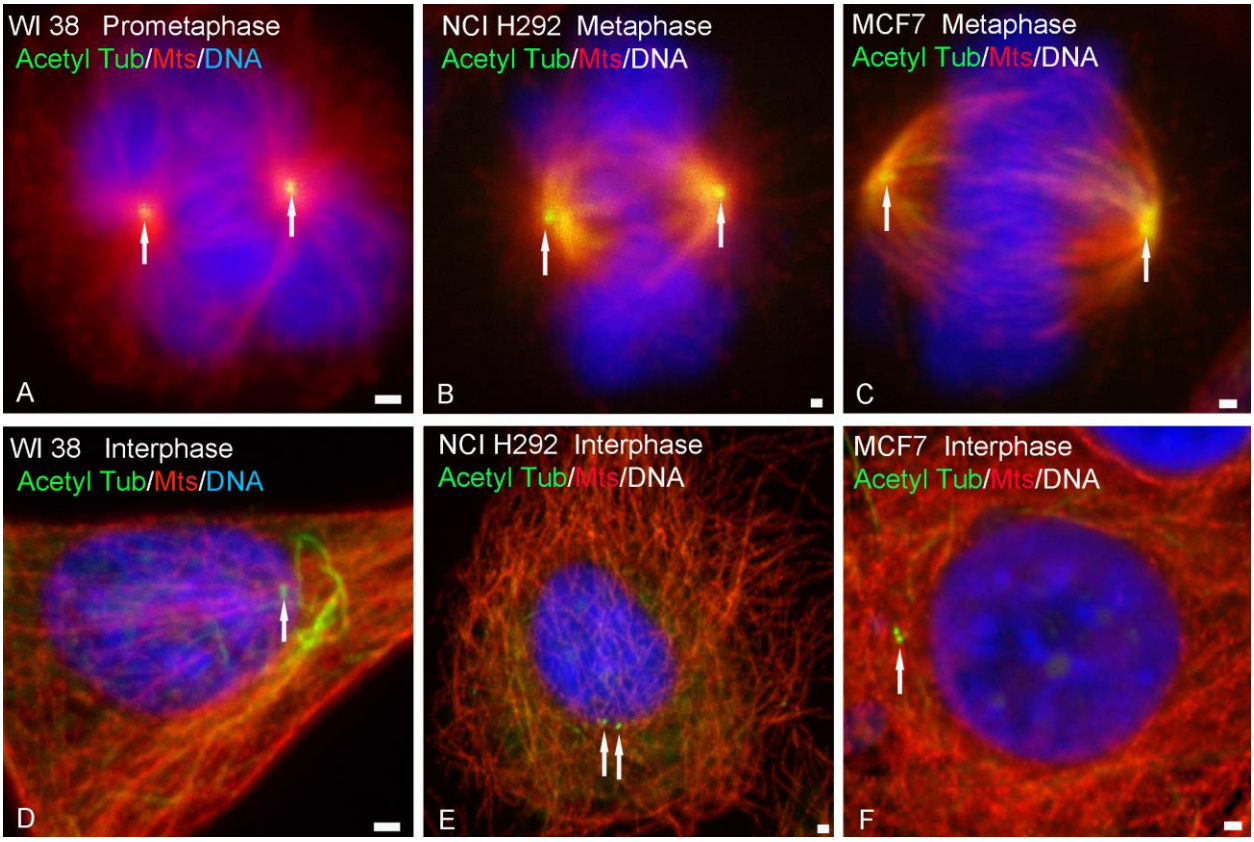

Figure 2. Detection of acetylated α-tubulin in control WI 38 and the cancer cell lines NCI H292 and MCF7. **A and D**: WI 38 lung fibroblast cell at prometaphase (A: blue, DNA) and interphase (D: blue, DNA) showing acetylated α-tubulin localized to the centrioles (A and D: green, arrows; red, microtubules). At interphase, a subset of the total cytoplasmic microtubules are detected with acetylated α-tubulin, residing near the paired interphase centrioles (D: green; arrow, centrioles). **B and E**: NCI H292 metaphase (B: blue, DNA) and interphase (E: blue, DNA) cells. At mitosis, acetylated α-tubulin is detected both at the spindle pole centrioles (B: green, arrows) and slightly stains the spindle pole microtubules. Fewer, sparse cytoplasmic microtubules are observed at interphase (E: green) with a pair of distinct centrioles detected (E: green, arrows). **C and F**: MCF7 breast cancer cells at metaphase (C: blue, DNA) and interphase. (F: blue, DNA). Acetylated α-tubulin is detected at the metaphase centrioles (C: green, arrows; blue, DNA) and spindle pole microtubules are slightly labeled. At interphase, acetylated α-tubulin is strongly detected at a pair of centrioles (F: green, arrows) adjacent to the nucleus (F: blue, DNA). Few cytoplasmic microtubules are labeled with acetylated α-tubulin. All images tripled-labeled for acetylated α-tubulin (green), microtubules (red) and DNA (blue). Bars= 1μM.

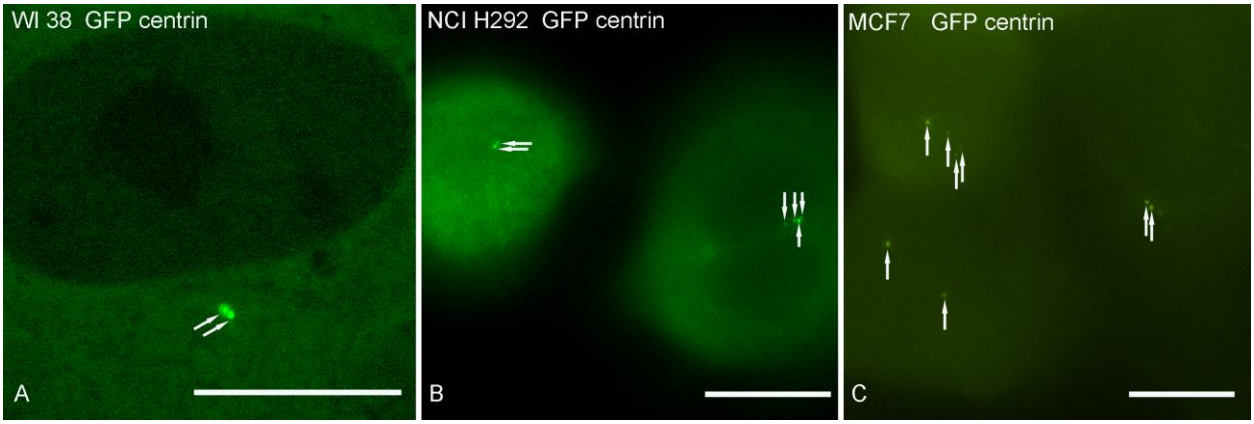

Figure 3. GFP centrin expression in control WI 38 lung fibroblast and two cancer cell lines, NCI H292 lung carcinoma and MCF7 breast cancer lines. A: WI 38 showing typical pair of centrioles as detected by GFP centrin construct expression (arrows). B: a pair of interphase NCI H292 cells showing a cell with a pair of centrioles (left image; double arrows) and another cell with four centrioles (right image; quad arrows). C: three interphase MCF7 breast cancer cells. The upper cell has four centrioles expressing GFP centrin (quad arrows), which are not tightly apposed in this cell. The far right hand cell has a pair of GFP centrin expressing centrioles tightly paired (dual arrows) while the bottom left cell has a pair of centrioles splitting apart (single arrows). This latter cell may be entering mitosis. Green: GFP centrin. Bars= 10µm.

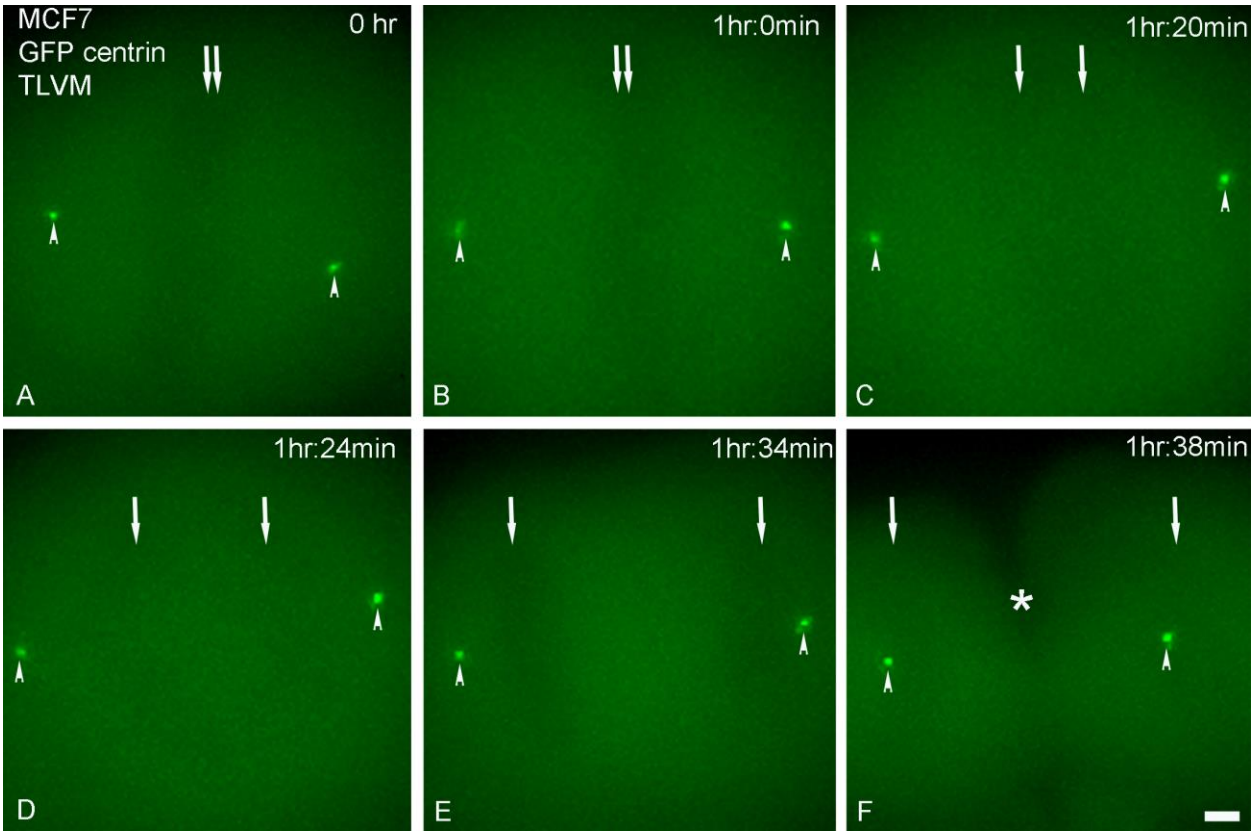

Figure 4. Time-lapse Video Microscopy (TLVM) of Mitotic GFP centrin MCF7 Breast Cancer Cell Line. A-B: metaphase stage, with chromosomes aligned at cell center (double arrows) and GFP centrin-expressing centrioles (green) at each spindle pole (arrowheads). C-F: normal karyokinesis (arrows) ensues over the next ~38 mins, resulting in cell division (F: * denotes cleavage furrow) as GFP-centrin expressing centrioles (green) remain clearly visible at the spindle poles (arrowheads). Upper right of each panel: hours: minutes post imaging. Bar=1µm.

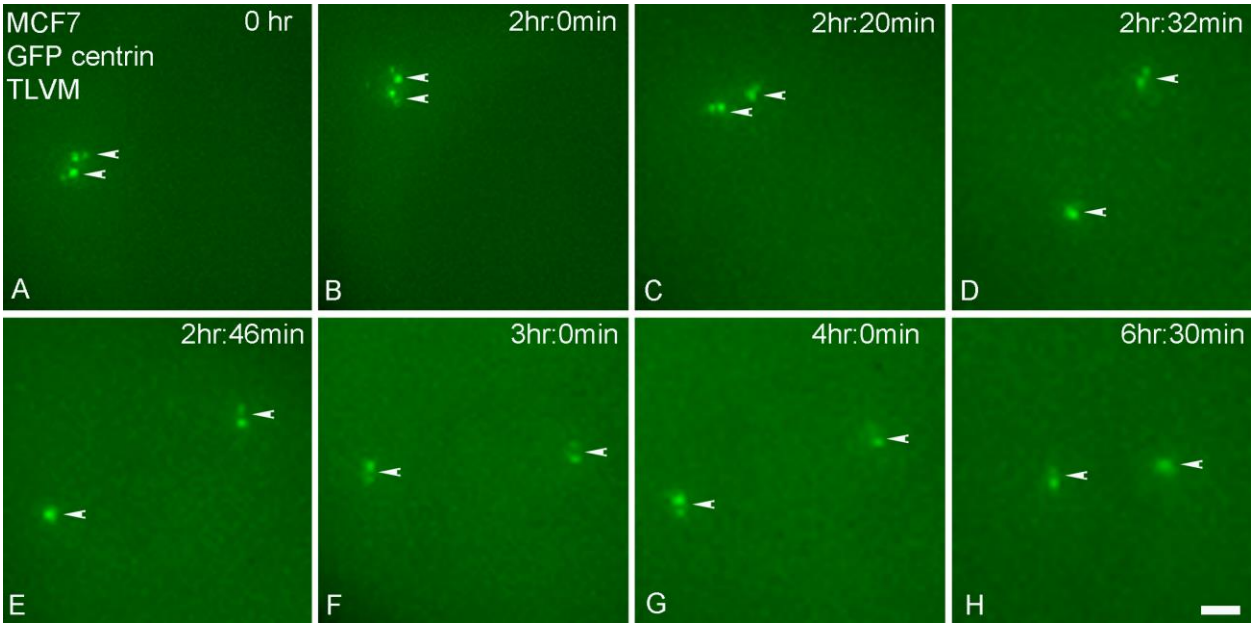

Figure 5. TLVM of MCF7 Breast Cancer Cells Expressing GFP centrin labeled centrioles. A: late interphase cell, with 4- GFP centrin expressing centrioles (green, arrowheads) visible in the cytoplasm. B: movement of the 4 centrioles in the cytoplasm occurs over the next 2 hrs. C-E: centriole splitting and migration (arrowheads) in the cytoplasm occurs rapidly just prior to mitosis onset (~26 min). F-G: mitosis following nuclear envelop breakdown. Note the centrioles pairs remain at their respective poles (arrowheads). H: cytokinesis failed after 2 hrs and the cell re-entered interphase. The centriole pairs moved towards the cell center following mitosis (arrowheads). Upper right of each panel: hours: minutes post imaging. Bar=1µm

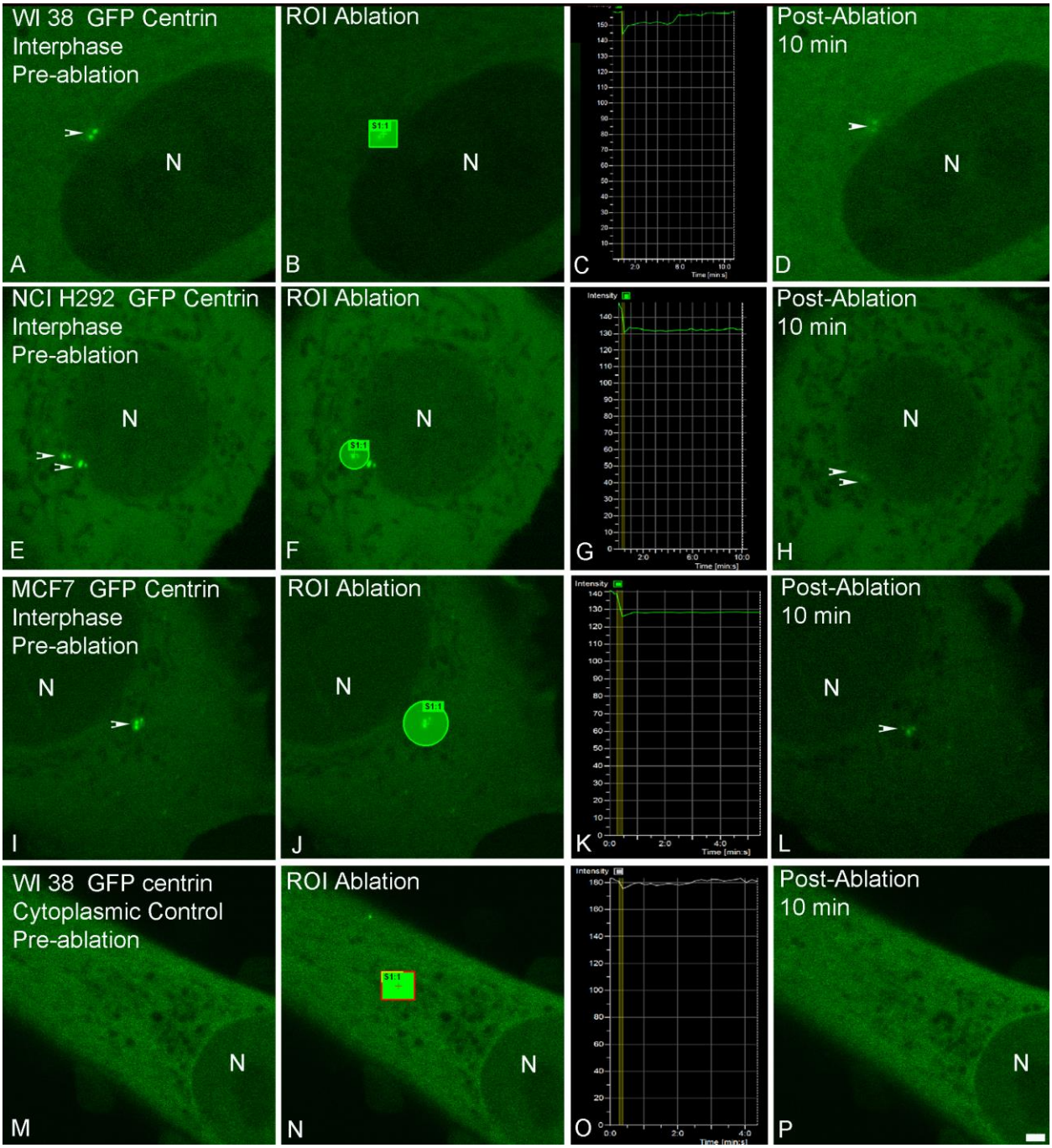

Figure 6. Dynamics of GFP Centrin Recovery following Fluorescent Recovery after Photobleaching (FRAP) in noncancerous and cancer cell lines. A-D: a WI38 noncancerous cell expressing GFP centrin at the centrioles (green; arrowhead). The centriole ablation region of interest (ROI) is shown in B (square box) while the full recovery of centriole GFP fluorescence over 10 min following laser ablation is depicted in C. The final appearance of GFP centrin-expressing centrioles after 10 min rescue is shown in D (arrowhead). E-F: NCI H292 cancer cell line with 4 centrioles observed by GFP centrin expression (E: arrowheads). After ablation of 1 centriole pair (F, circle), very little fluorescence recovery is observed over the following 10 minutes (G). H: centrioles expressing GFP centrin after 10 minutes rescue from ablation (arrowheads). Note that both centriole pairs demonstrate reduced GFP fluorescence suggesting

cancer centrioles are less stable structures than controls. I-L: MCF7 breast cancer cell. Four centrioles are observed by GFP centrin fluorescence (I: arrowhead). All 4 centrioles were targeted for ablation (J, circle). Like the results for the NCI H292 cancer cell line, FRAP analysis indicated very little (<20%) rescue of GFP centrin in the centrioles over the subsequent 5 min (K). Final appearance of the GFP centrin centrioles post 5 min recovery is shown in L (arrowhead). M-N: a control WI 38 noncancerous cell expressing GFP centrin in the cytoplasm (M). A non-centriole region was selected for ablation (N: square box) and the full recovery of cytoplasmic fluorescence is shown employing the same settings as for WI 38 in the top panel (O; P: fluorescence image 5 min post ablation. N=nucleus. Bar=1µm**.**

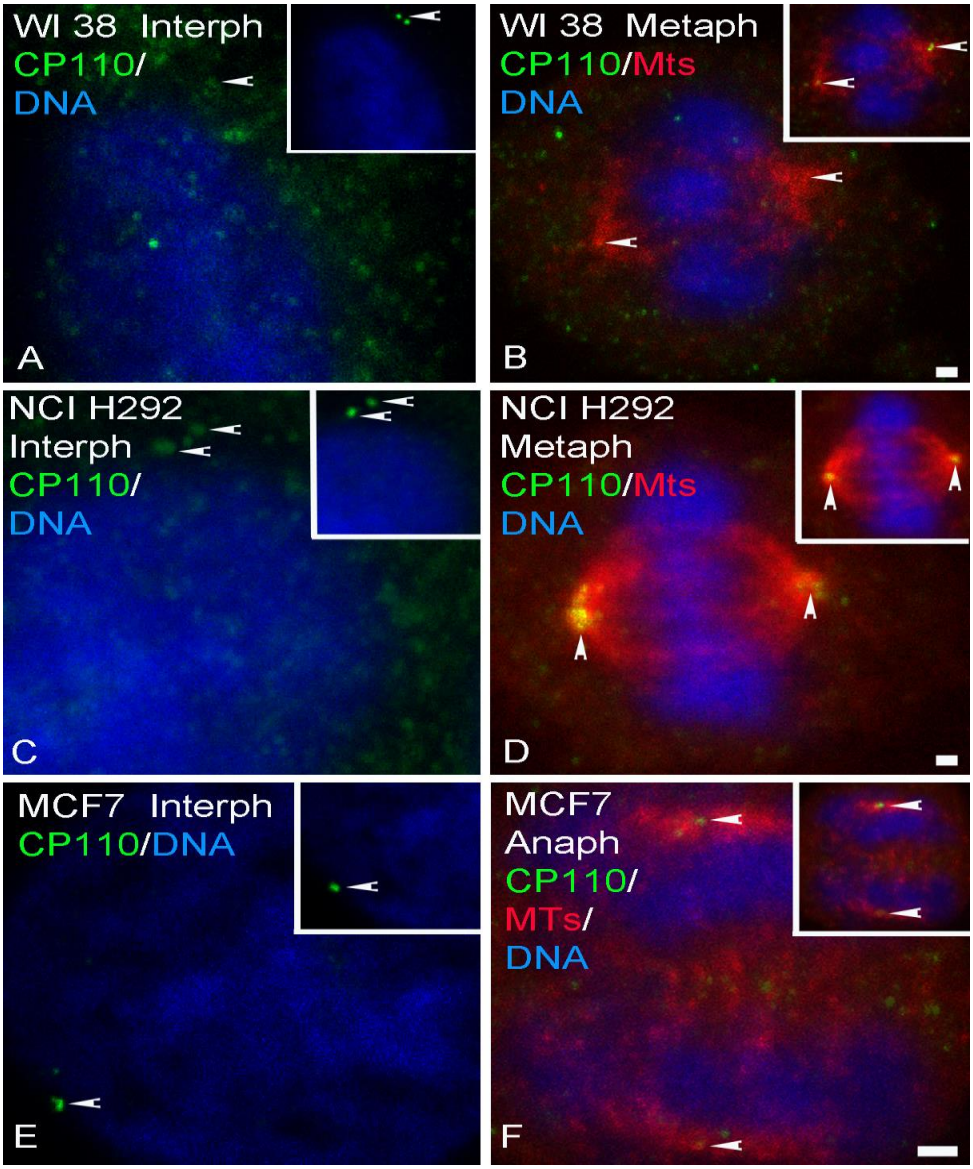

Figure 7. Detection of the Centrosomal Marker CP 110 is higher in Cancer Cells. **A-B**: In WI 38 lung fibroblast, CP 110 is only weakly expressed at the centrosomes in interphase (A: green, arrowhead; blue, DNA) and metaphase cells (B: green, arrowheads; red, microtubules; blue, DNA). Insets: corresponding centrin 20H5 detection at the centrioles (green, arrowheads) in the same cells. **C-D**: NCI H292 lung carcinoma cells demonstrating stronger detection of CP 110 at the centrosomes at interphase stage (C: green, arrowheads; blue, DNA) and metaphase cell (D: green, arrowheads; red, microtubules; blue, DNA). Insets: corresponding detection of centrosomes with pericentrin 28144 antibody (green, arrowheads; red, microtubules; blue, DNA). **E and F**: In the breast cancer cell line MCF7, CP 110 is also strongly detected at interphase centrosomes (E: green, arrowhead; blue, DNA) and the mitotic spindle pole centrioles (F: green, arrowheads; red, microtubules; blue, DNA). Insets: pericentrin 28144 antibody detection at the centrosomes (arrowheads). All images quadruple-labeled for CP110 (green), microtubules (red), DNA (blue) and either centrin 20H5 (A-B; color assigned green) or pericentrin 28144 (C-F; color assigned green). Bars= 1µm.

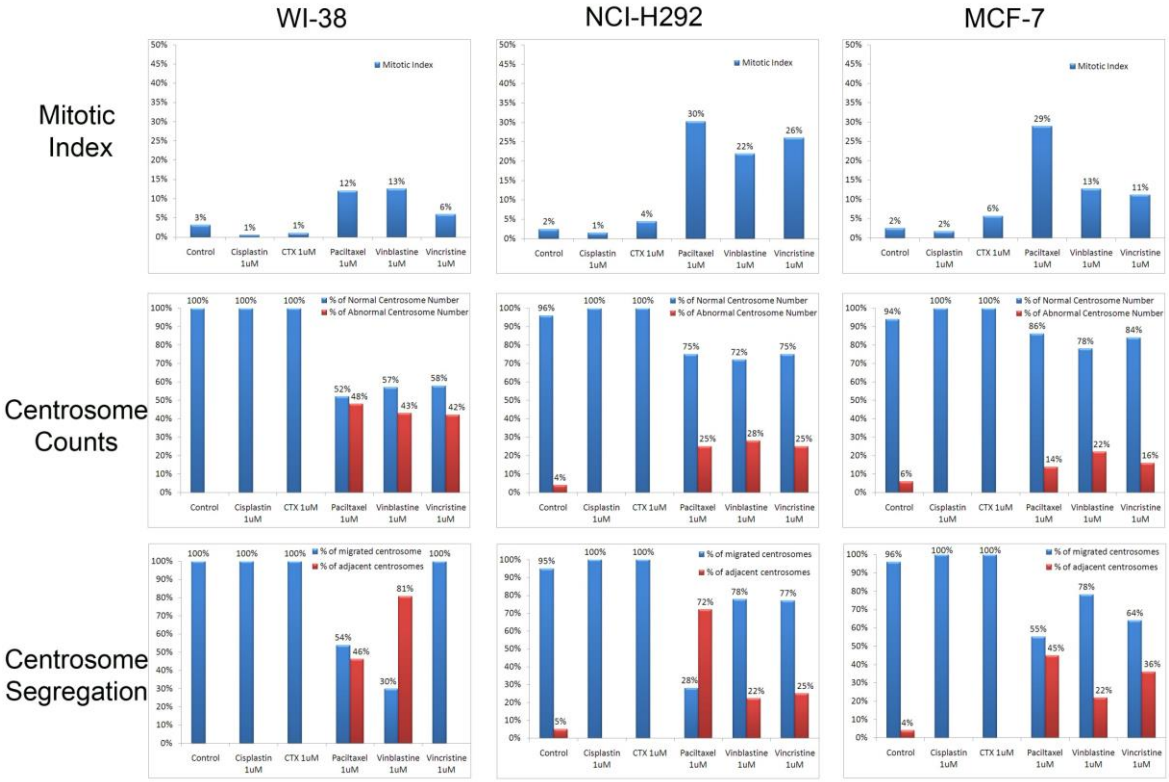

Figure 8. Graphic analysis of mitotic index, centrosome numbers and position in WI-38, NCI-H292 and MCF-7 cells after chemotherapeutic drug treatments. Top graphs: cell cycle arrest in mitosis is significantly increased in WI-38 lung fibroblast, NIC-H292 lung carcinoma, and MCF-7 breast cancer lines after treatment with 1µM paciltaxel, vinblastine sulfate or vincristine sulfate, but not cisplatin or cyclophosphamide (CTX). Middle graphs: normal detection of two centrosomes is significantly disrupted in all three cell lines after 1µM paciltaxel, vinblastine sulfate or vincristine sulfate but not cisplatin or CTX. Abnormal centrosome numbers include 1, 3, 4 or more cytoplasmic centrosomes as detected by anti-pericentrin antibody. Bottom graphs: centrosome segregation is dramatically blocked in NCI-H292 and MCF-7 cancer cell lines after 1µM paciltaxel, vinblastine sulfate or vincristine sulfate treatment but not cisplatin or CTX. Curiously, for the normal lung fibroblast line WI-38, 1µM vincristine did not show inhibition of centrosome splitting despite complete microtubule disassembly. WI-38 cells treated with 1µM paciltaxel and vinblastine sulfate did show significant effects on centrosome position within the cytoplasm.

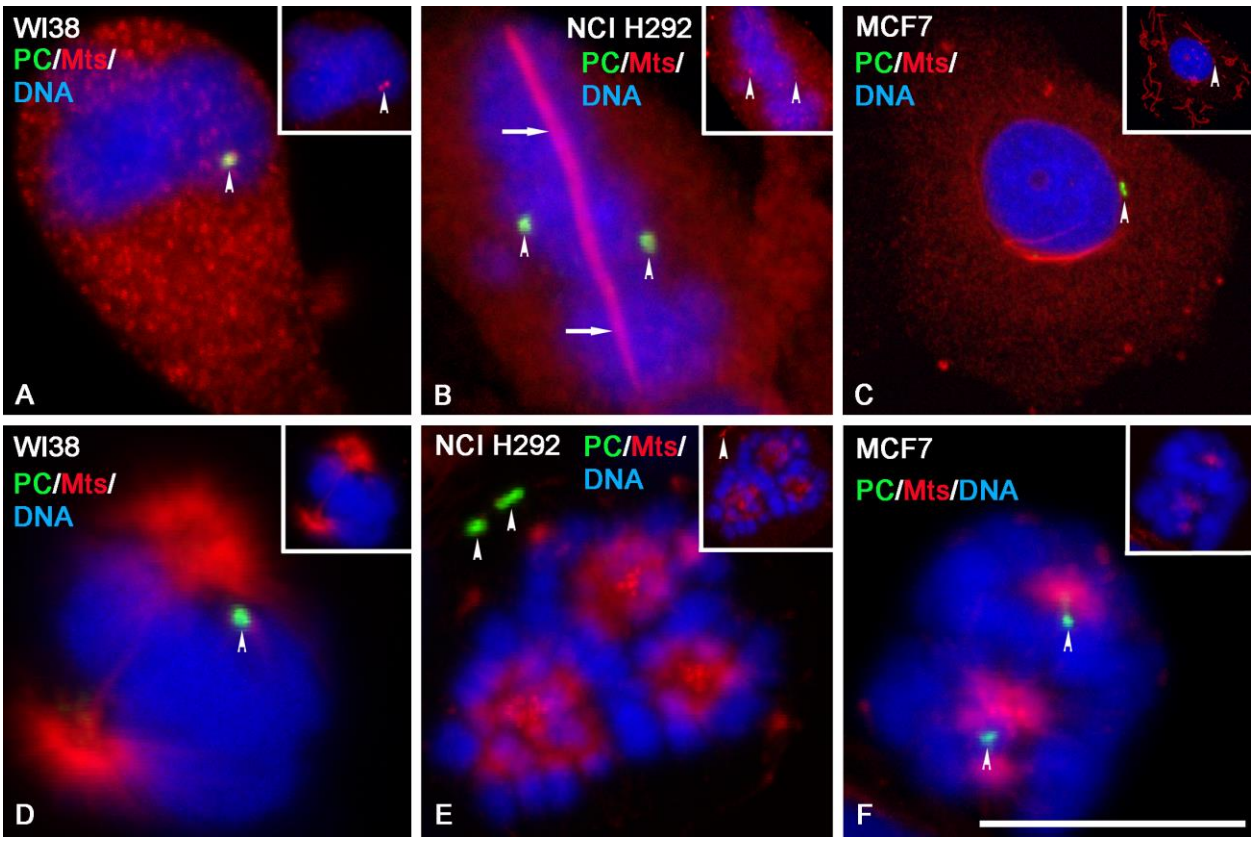

Figure 9. Pericentrin and acetylated α-tubulin in WI38, NCI-H292 and MCF7 cells after 1 μM vincristine sulfate (A-C) or paciltaxel (D-F) exposure for 24hrs. A-C: Cytoplasmic microtubules disassemble in the presence of vinblastine sulfate (red; blue, DNA) except for paracrystalline arrays found largely in the NCI- H292 fibroblast carcinoma cells (B: red, arrows). Pericentrin labels single (A: green, arrowhead) or duplicated centrosomes (B-C: green, arrowheads) in all 3 cell lines. Insets: acetylated α-tubulin (red, arrowheads; blue, DNA) detects centrioles in absence of assembled microtubules. D-F: Paciltaxel greatly augments spindle microtubule assembly (red; blue, DNA) while affecting centrosome numbers (A: green, arrowhead) and cytoplasmic position in most cancer cell lines (E-F: green, arrowheads). Insets: acetylated α-tubulin (red) after paciltaxel treatment detects mostly microtubules and some centrioles (E: inset, arrowhead). All images quadruple-labeled for pericentrin 4448 (green), general microtubules (red), DNA (blue) and acetylated α-tubulin (Cy5 antibody labeled and color assigned red). Bar=10μm.

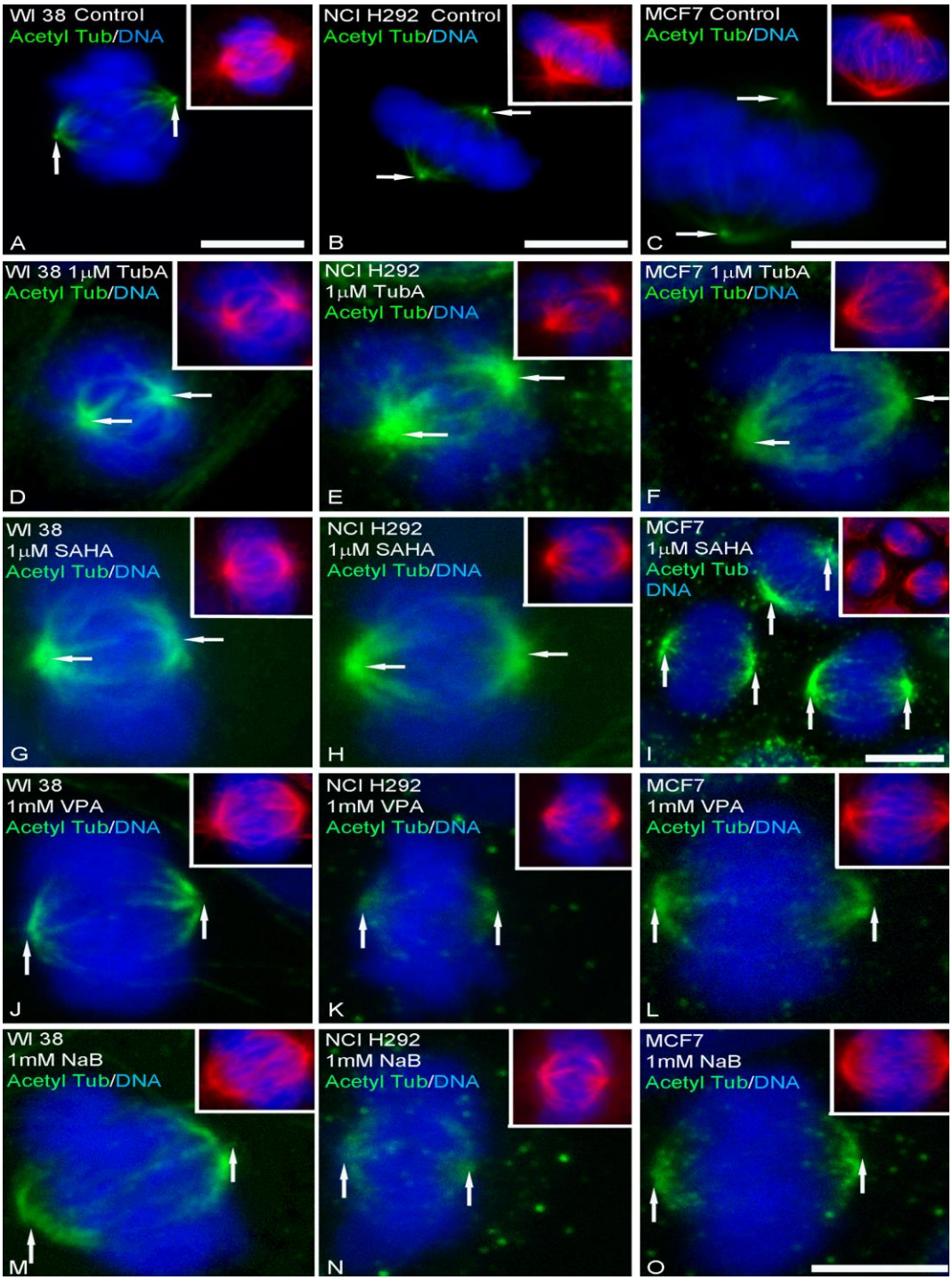

Figure 10. HDAC6$_i$ causes Loss of Centriolar Acetylation and Increases Spindle Microtubule Acetylation in Cancer Cells. **A-C**: non-drug treated mitotic metaphase cells showing acetylated α-tubulin localized to the centrioles (A-C: green, arrows; blue, DNA) in WI 38 (left panels), NCI H292 (middle panels), and MCF7 cells (right panels). Acetylated α-tubulin is also weakly expressed at the spindle poles relative to total microtubules (insets: red, microtubules; blue, DNA). **D-E**: cells treated with 1μM Tubastatin A HDAC6i for 48 hrs prior to fixation. Acetylated α-tubulin is lost at the centrioles (D-F: green, arrows) and a dramatic increase in

spindle microtubule acetylation is observed (D-F: green) compared to non-drug treated controls. Insets: microtubules and DNA. **G-I**: cells treated with 1 µM SAHA show similar loss of centriole acetylated α-tubulin and increased spindle microtubule hyperacetylation (G-I: green, arrows; blue, DNA; insets: red, microtubules; blue, DNA). **J-L**: the non-HDAC6i valproic acid (VPA; 1mM; 48 hrs) causes loss of centriole aceytlation (J-L: green, arrows) but does not increase hyperacetylation of spindle microtubules. Insets: red, microtubules; blue, DNA. **M-O**: the non-HDAC6i sodium butyrate (NaB; 1mM; 48hrs) also causes the loss of centriole acetylation but does not initiate spindle pole hyperacetylation (M-O: green, arrows; blue, DNA; insets: red, microtubules; blue, DNA). All images quadruple-labeled for Δ-2 tubulin (not shown), microtubules (red), DNA (blue) and acetylated α-tubulin (cy5 labeled and color assigned green). Bars=1µm**.**

Table 1. Detection of Centriole and Centrosome Markers in WI38, NCIH292 and MCF7 Cells.

Antibody	Predicted Localization/ Function	WI 38		NCI H292		MCF7	
		Interph	Mitosis	Interph	Mitosis	Interph	Mitosis
Cep135	Centriole/ assembly	+++	+++	+++	+++	+++	+++
HsSAS-6	Centriole/ cartwheel assembly	+++	+++	ND	ND	+++	+++
CP110	Centriole/ duplication	+	+	+++	+++	+++	+++
C-Nap1	Centriole/ parental centriole cohesion	+	-	-	-	-	-
Ninein	Centriole/ MT capping and centriole anchoring	-	++	-	++	-	++
Cenexin 1	Centriole/ scaffold protein	+++	++	+++	++	+++	++
Delta (Δ)-2 Tubulin	Centrioles/ PTM	+++	+++	+++	+++	+++	+++
Centrin 20H5	Centrioles/calcium binding protein	++	++	++	++	++	++
CEP192	Centrosome/ MT-nucleating scaffold protein	+	+++	ND	ND	+	++
Pericentrin	Pericentriolar material/ scaffold	++	+++	++	+++	++	+++
γ-Tubulin	Pericentriolar material/ MT nucleation	++	+++	++	+++	++	+++
NuMA	Nucleus or spindle poles/ focus MT minus ends at mitosis	+++	+++	+++	+++	+++	+++
Acetylated α-tubulin	Centrioles and MTs/ PTM.	++	+++	-	++	-	++
Tyros 1A2	Tyrosinated MTs/PTM	+++	+++	+++	+++	+++	+++

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X_ No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics

_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X__No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and

an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____X_____No_____

If yes, please describe your plans:

The discoveries made here are being analyzed and confirmed. Once that work is completed, then they will be prepared for publication in a prominent cell and cancer biology journal.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention,

diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
YesNo

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
YesNo
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? YesNo

If yes, how many licenses were granted?

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Calvin Simerly, PhD	Research Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login)	
CSIMERLY	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Pensacola Junior College Pensacola, Florida	A.A.	1974-1976	Biology
Florida State University Tallahassee, Florida	B.S.	1976-1979	Biological Science
University of Wisconsin Madison, Wisconsin	Ph.D.	1995-1998	Endocrinology-Reproductive Sciences

A. POSITIONS AND HONORS

Positions (since 1997)
1997-1998: Division of Reproductive SciencesStaff Scientist I Oregon Regional Primate Research Center Beaverton, Oregon
1998-2001: Division of Reproductive SciencesStaff Scientist II Oregon Regional Primate Research Center (Assistant Professor Beaverton, OregonEquivalent)
1999-2001: Department of Obstetrics/GynecologyResearch Assistant Oregon Health & Science University, Professor Portland, Oregon
2001-2008: Department of Obstetrics/GynecologyAssociate Professor & Reproductive Sciences, University of Pittsburgh Pittsburgh, Pennsylvania
2008-Present Department of Obstetrics/GynecologyResearch Associate Professor & Reproductive Sciences, University of Pittsburgh Pittsburgh, Pennsylvania

Honors
1986-present American Society for Cell Biolog
2005-presentInternational Society for Stem Cell Research

B. SELECTED PEER-REVIEWED PUBLICATIONS (from 1999)

1. Hewitson L, Dominko T, Takahashi D, Ramalho-Santos J, Sutovsky P, Fanton J, Jacob D, Monteith D, Neuringer M, Battaglia D, Simerly C, Schatten G. Births of ICSI monkeys: Unique checkpoints during the first cell cycle of fertilization. Nat Medicine 5(4):431-433, 1999.
2. Simerly C, Zoran SS, Payne C, Dominko T, Sutovsky P, Navara CS, Salisbury JL, Schatten G: Biparental inheritance of γ-tubulin during human fertilization. Molecular reconstitution of functional zygotic centrosomes in inseminated human oocytes and in cell-free extracts nucleated by human sperm. Mol Biol Cell 10(9):2955-2969, 1999.
3. Mountain V, Simerly C, Howard L, Ando A, Schatten G, Compton D. The kinesin-related protein, HSET, opposes the activity of Eg5 and cross links microtubules in the mammalian mitotic spindle. J Cell Bio 147(2):351-365, 1999.
4. Sutovsky P, Moreno R, Ramalho-Santos J, Dominko T, Simerly C, Schatten G. Ubiquitin tag for sperm mitochondria. Nature 402:371-372, 1999.
5. Chan AWS, Dominko T, Takahashi D, Simerly C, Schatten G. Clonal propagation of primates by embryo splitting. Science 277(5451):317-321, 2000.

6. Simerly C, Dominko T, Navara C, Payne C, Capuano S, Gosman G, Chong KY, Takahashi D, Chace C, Compton D, Hewitson L, Schatten, G. Molecular Correlates of Primate Nuclear Transfer Failures. Science 300: 297, 2003. PMID: 12690191.
7. Simerly C, Navara CS, Hyun SH, Lee BC, Kang SK, Capuano S, Gosman G, Dominko, T, Chong K Y, Compton D., Hwang, WS, & Schatten G. Embryogenesis and blastocyst development after somatic cell nuclear transfer in nonhuman primates: overcoming defects caused by meiotic spindle extraction. Dev Biol 276: 237– 252, 2004. PMID: 15581862.
8. Navara CS, Mich-Basso JD, Redinger CJ, Ben-Yehudah A, Jacoby E, Kovkarova-Naumovski E, Sukhwani M, Orwig K, Kaminski N, Castro CA, Simerly CR, Schatten G. Pedigreed Primate Embryonic Stem Cells, Express Homogeneous Familial Gene Profiles. Stem Cells 25(11):2695-2704, 2007. PMID: 17641389.
9. Simerly CR, Navara CS, Castro CA, Turpin JC, Redinger CJ, Mich-Basso JD, Jacoby ES, Grund KJ, McFarland DA, Oliver SL, Ben-Yehudah A, Carlisle DL, Frost P, Penedo C, Hewitson L, Schatten GP. Establishment and Characterization of Baboon Embryonic Stem Cell Lines: An Old World Primate Model for Regeneration and Transplantation Research. Stem Cell Research. Stem Cell Res. 2: 178-187, 2009. PMID: 19393591.
10. Simerly CR, Castro CA, Jacoby E, Grund K, Turpin T, McFarland D, Champagne J, Jimenez, Jr JB, Frost P, Bauer C, Hewitson L, Schatten G. Assisted Reproductive Technologies (ART) with Baboons Generate Live Offspring: A Nonhuman Primate Model for ART and Reproductive Sciences. Reprod Sci. Jul 16, 2010. [Epub ahead of print]. PMID: 20631291. PMCID Pending.
11. Ben-Yehudah A, Easley IV CA, Hermann B, Castro C, Simerly C, Orwig K, Mitalopov S, Schatten G. System Biology Discoveries Using Non-Human Primate Pluripotent Stem and Germ Cells: Novel Gene and Genomic Imprinting Interactions As Well As Unique Expression Patterns. Stem Cell Research and Therapy. Stem Cell Res Ther., Aug 5:1(3):24, 2010. PMID: 20699013
12. Simerly, C., McFarland, D., Castro, CA, Lin, C-C, Redinger, C., Jacoby, E., Mich-Basso, J., Orwig, K., Mills, P., Ahrens, E., Navara, C. and Schatten, G. (2011). Interspecies Chimera Between Nonhuman Primate (NHP) Embryonic Stem Cells (ESCs) and Mouse Embryos: Rhesus and Baboon ESCs Engraft in Murine Morulae and Blastocysts, but Do Not Contribute to Cellular Lineages After Implantation. Stem Cell Res. 7:28-40.

C. ACTIVE RESEARCH SUPPORT

1R25CA163168-01 (Schatten, G.) 12/1/11–11/30/16 3.6 calendar

NIH - Frontiers in Stem Cells in Cancer

This training course offers annual Cancer Education short-courses as well as limited support for subsequent pilot research projects and on-going mentoring.

D. PERSONAL STATEMENT. My overall interest lie in the Cell Biology of Fertilization and Preimplantation Development with particular interest in understanding centrosome inheritance and function as related to motility mechanisms during fertilization, cytoskeletal dynamics, meiosis and mitosis (chromosome segregation and non-disjunction), somatic cell nuclear transfer (cloning) and stem cells (embryonic and induce pluripotent ESCs). My fundamental research investigations have importance to the fields of Molecular Medicine (infertility; contraception; birth defects; mental retardation; human development), Stem Cell Potentials (molecular biology of the cytoskeleton; ionic and molecular controls of intracellular regulation; cytoplasmic and nuclear reprogramming; cell cycle progress; extranuclear inheritance) and Gene Therapy (transgenic disease models; nonhuman reproductive cloning).