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:** Temple University – of the Commonwealth System of Higher Education

2. **Reporting Period (start and end date of grant award period):** 1/1/2010-12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Germaine A Calicat, MA

4. **Grant Contact Person's Telephone Number:** 215-204-7655

5. **Grant SAP Number:** 4100050909

6. **Project Number and Title of Research Project:** Project 11: Role of the B55α Protein Phosphatase 2A Holoenzyme in Modulating the Phosphorylation of p107 and Related Proteins

7. **Start and End Date of Research Project:** 12/1/2010 – 8/31/2011

8. **Name of Principal Investigator for the Research Project**: Xavier Graña, Ph.D.

9. **Research Project Expenses**.

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

   $45,600

   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, First Name	Position Title	% of Effort on Project	Cost
Kurimchak, Alison	Graduate Research Assistant	100%	$30,000

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, First Name	Position Title	% of Effort on Project
Graña, Xavier	Principal Investigator	10%

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

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__X____ No_____

If yes, please indicate the source and amount of other funds:
Bridge Funding from Temple University and NIH grant MH083585 to Xavier Graña: ~$15,000

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:
Elucidation of a novel mechanism of haploinsufficient tumor suppression in Prostate Cancer.	☐NIH ☐ Other federal (specify:_____ _____) ☒ Nonfederal source (specify: WW. Smith Charitable Trust Grant)	June 2013	$100,000	$100,000
Reactivation of p107 by PP2A in chondrocyte maturation and cell cycle exit: a paradigm for PP2A dependent rapid activation of pocket proteins	☐NIH ☒ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	November 1013	$621,358	Pending
Evaluation of PPP2R2A as haploinsufficient tumor suppressor in prostate cancer	☒NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify:	June 2013	$426,250	Preparing resubmission (40% percentile)

	_____)			
Protein Phosphatase 2A role in activation of the retinoblastoma protein family	☒NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	November 2011	$1,385,755	Not funded

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:

A resubmission of the R21 listed above in July or November 2014
A new NSF application on the Determinants of substrate specificity of the B55alpha/PP2A holoenzyme in November 2014.

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

Plans for the future of this research project include obtaining federal funding for continuation of the work described here and/or for two spin-offs that have resulted from this work (see answer to question 11). Also, as a result from this work, I have obtained a 2-year Temple University/Fox Chase Cancer Center Nodal Award to extend our preliminary data on one spin-off on "Mechanisms of PPP2R2A Tumor Suppression in Prostate Cancer" in collaboration with Vladimir Kolenko from FCCC.

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___X___ No_____

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

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female		1	1	
Unknown				
Total		**1**	**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic		1	1	
Unknown				
Total		**1**	**1**	

	Undergraduate	Masters	Pre-doc	Post-doc
White			1	
Black				
Asian		1		
Other				
Unknown				
Total		**1**	**1**	

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.

    The funding of the graduate student stipend for a year allowed an expansion of this project, which was at a preliminary stage and needed additional funding to generate sufficient preliminary data for submission of an application to a federal agency. This came at a time when funding for my lab was limited, especially for this project. The project progressed over that year very significantly and laid out the foundation for completion of the thesis of the Graduate student, who will defend at the end of this semester. It also led to the publication of a high quality paper in Molecular and Cellular Biology, a figure in a paper in the Journal of Biological Chemistry, the publication of two invited review articles on the topic and the preparation of various federal and non-federal grant applications. One of these has been funded, as well as a Temple/FCCC Nodal award, and two federal grants are pending or in resubmission and new grants are planned. In addition, I also was invited and served as editor of a monograph review issue in Genes and Cancer. All this has enhanced the research quality and capacity of my laboratory, our graduate program and, in turn our Research Institute.

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___X____ No_____

If yes, please describe the collaborations:

Raymond J. Deshaies, Howard Hughes Medical Institute, California Institute of Technology, 1200 E California Blvd. Pasadena CA

Michael J. Sweredoski and Sonja Hess from the Proteome Exploration Laboratory, Beckman Institute, California Institute of Technology.

Shufang Wu and Francesco De Luca from Section of Endocrinology and Diabetes, St. Christopher's Hospital for Children, Department of Pediatrics, Drexel University College of Medicine, Philadelphia, Pennsylvania.

Dale S. Haines and Judit Garriga from my same Institute.

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___X____ No_____

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

The NSF application that is pending includes a new program for the training of students from the magnet, Blue Ribbon Masterman High School during 5-6 weeks in the summer. One Masterman student has been recruited to start this summer. If the grant is funded two students will be trained each summer.

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

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims <u>for the period that the project was funded (i.e., from project start date through end date).</u> 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.

Aims of the original proposal

The retinoblastoma family of growth suppressor proteins consists of the tumor suppressor pRB and the functionally and structurally related proteins p107 and p130. p107 and p130 have been implicated in tumorigenesis in mouse knockout models where either p107 or p130 are simultaneously knocked out together with ablation of a pRB allele. These compound mice exhibit tumor patterns that are distinct from those seen in pRB heterozygous mice. Also, simultaneous inactivation of p107/p130 has shown that these proteins are critical for cell cycle exit in chondrocytes during development and for endochondral bone formation. When hypophosphorylated, pocket proteins bind and inactivate the E2F family of transcription factors, which are required for cell cycle regulated gene expression. These functions of pocket proteins are inactivated via phosphorylation by CDKs. CDKs are activated in mid to late G1 upon mitogenic stimulation of hyperphosphorylating pocket proteins. This results in disruption of their association with E2F transcription factors and other proteins. We have previously shown that PP2A plays a role in restricting the phosphorylation of pocket proteins through the cell cycle, which results in their rapid dephosphorylation when the activity of CDKs is compromised (Garriga et al., 2004). PP2A

enzymes are Ser/Thr protein phosphatases that consist of a catalytic and a scaffold subunit that complex to a regulatory B subunit, which is responsible for substrate specificity and subcellular localization. There are four different families of B subunits, each with multiple members (reviewed in (1,2). We have recently identified a trimeric holoenzyme that dephosphorylates p107 and p130. B55α is the regulatory subunit of this trimeric holoenzyme. We have shown direct association of B55α with p107, and dephosphorylation of p107 by the trimeric holoenzyme *in vitro*. We have found that overexpression of B55α induces p107 dephosphorylation while its knockdown induces hyperphosphorylation (Jayadeva et al., 2010, in revision when the project started). **The aims of this study are:**

(1) To determine if endogenous p107 interacts with endogenous B55α in a variety of cells and/or upon certain stimuli.

(2) To identify domains in B55α critical for binding to p107.

(3) To determine if B55α is required for FGFα-induced dephosphorylation of p107 in chondrocytes.

All the aims were completed approximately within one year of submission of the application. Aims 1 and 3 are part of a manuscript published in MCB (Kurimchak et al., 2013) and will be described first. Aim 2 was a more concrete aim that was included in a manuscript that was under review after the application was submitted (Jayadeva, et al., 2010), and will be described separately.

Aims 1 and 3:
Aim 1 is described in Figs 1-3 of Kurimchak et al (2013).
Aim 3 is described in Fig 4 of Kurimchak et al (2013).

The remainder figures in this paper were generated following completion of the first year and are not described.

Summary of published work (from Kurimchak et al., 2013)(3)

The phosphorylation state of pocket proteins during the cell cycle is determined at least in part by an equilibrium between inducible CDKs and the serine/threonine protein phosphatase PP2A. Two trimeric holoenzymes consisting of the core PP2A catalytic/scaffold dimer and either the B55α or PR70 regulatory subunits have been implicated in the activation of p107/p130 and pRB, respectively. While the phosphorylation state of p107 is very sensitive to forced changes of B55α levels in human cell lines, regulation of p107 in response to physiological modulation of PP2A/B55α has not been elucidated. Here we show that FGF1, which induces maturation and cell cycle exit in chondrocytes, triggers rapid accumulation of p107-PP2A/B55α complexes coinciding with p107 dephosphorylation. Reciprocal solution-based mass-spectrometric analysis identified the PP2A/B55α complex as a major component in p107 complexes, which also contain E2F/DPs, DREAM subunits and/or cyclin/CDK complexes. Of note, p107 is one of the preferred partners of B55α, which also associates with pRB in RCS cells. FGF1 induced dephosphorylation of p107 results in its rapid

accumulation in the nucleus, formation of larger complexes containing p107, and enhances its interaction with E2F4 and other p107 partners. Consistent with a key role of B55α in the rapid activation of p107 in chondrocytes, limited ectopic expression of B55α results in marked dephosphorylation of p107, while B55α knockdown results in hyperphosphorylation. More importantly, knockdown of B55α dramatically delays FGF1 induced dephosphorylation of p107 and slows down cell cycle exit. Moreover, dephosphorylation of p107 in response to FGF1 treatment results in early recruitment of p107 to the *MYC* promoter, an FGF1/E2F regulated gene. Our results suggest a model where FGF1 mediates rapid dephosphorylation and activation of p107 independently of the CDK activities that maintain p130 and pRB hyperphosphorylation for several hours post p107 dephosphorylation in maturing chondrocytes.

Results

Aim 1. To determine if endogenous p107 interacts with endogenous B55α in a variety of cells and/or upon certain stimuli.

A model of chondrocyte maturation and cell cycle exit in response to treatment with FGFR3 ligands has been extensively characterized (4,5). RCS cells undergo cell cycle arrest upon treatment with FGF1 (4-7). As shown in Fig. 1A, RCS cells were treated with FGF1 plus heparin or heparin alone at the indicated time points, collected, stained with propidium iodide and subjected to FACS analysis. A transient accumulation of cells with G2/M DNA content is observed early and subsequently is reduced as cells accumulate with G0/G1 DNA content. The transient arrest at the G2/M transition has previously been shown to be caused by the transient inactivation of Cyclin B1/CDK1 complexes following FGF1 stimulation (8). Note that heparin control treated cells show a much smaller increase in the percent of cells with a G0/G1 DNA content, but the cells continue to proliferate and do not exit the cell cycle. Western blot analysis of lysates of FGF1 treated cells also confirmed the rapid dephosphorylation of p107 one hour after FGF1 treatment, which precedes pRB and p130 dephosphorylation by several hours (Fig. 1B) (9). As we have previously shown that B55α, a PP2A regulatory subunit belonging to the B family, modulates the phosphorylation state of p107 in human cells and p107 phosphorylation is very sensitive to forced changes in B55α expression, we determined B55α protein levels during the time course (Fig. 1B). B55α protein levels are unaffected by FGF1 treatment, while Collagen II, a marker of immature or undifferentiated chondrocytes, gradually decreases. Interestingly, an increase in p21 expression is observed around 10-15 hours post treatment coinciding with dephosphorylation of pRB and accumulation of hypophosphorylated p130. The upregulation of p21 in response to FGF1 stimulation in these cells has been reported earlier and coincides with inactivation of Cyclin E/CDK2 complexes and pRB hypophosphorylation (6). Since the CDK4/6 inhibitor p16 is also upregulated in response to FGF treatment in these cells (9), dephosphorylation pRB and p130 is likely a consequence of inhibition of CDK2 and CDK4, while dephosphorylation of p107 is compatible with upregulation of PP2A activity towards p107 as previously reported (10).

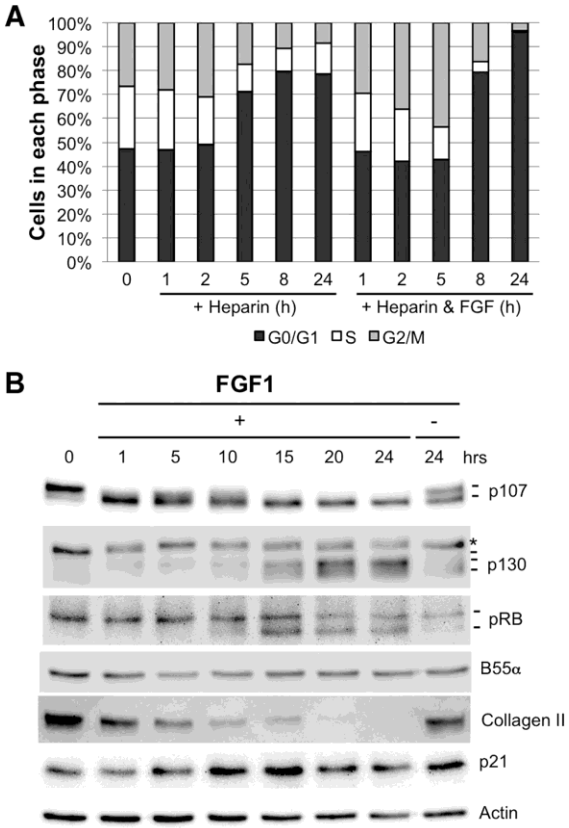

Fig 1. FGF1 induces rapid p107 dephosphorylation prior to pRB dephosphorylation, p130 accumulation, and subsequent G1 arrest and maturation in RCS cells without changes in the expression of B55α. RCS cells were stimulated with FGF1 in the presence of heparin or with heparin alone (control). Cells were collected at the indicated times for cell cycle FACS analysis (A) and western blot (B). Hyper and hypophosphorylated forms of p107/p130/pRB and maturation markers are indicated. * indicates a cross-reacting band (Kurimchak et al, 2013).

Kolupaeva *et al.* had reported that FGF1 induces transient formation of p107-PP2A/A-PP2A/C complexes in response to FGF1 treatment (10). In this setting, expression of adenovirus E4orf4, which primarily targets subunits of the B55 family (11), blocked FGF-mediated dephosphorylation of p107 (10). While we did not observe modulation of B55α levels by FGF1, it was still conceivable that FGF1 induces complex formation between the PP2A/B55α holoenzyme and p107 via some other mechanism. First, we determined if these complexes are detected in untreated RCS cells, as we have previously detected low levels of these complexes in U2-OS cells. Reciprocal immunoprecipitations with antibodies to p107 and B55α demonstrate the interaction between endogenous p107 and the three subunits of the PP2A/B55α holoenzyme in untreated cells (Fig. 2A). Given that p107 is rapidly dephosphorylated in RCS cells following FGF1 treatment, we next wanted to determine if this signal recruits additional PP2A/B55α holoenzymes to p107. RCS cells were treated with FGF for 30 minutes (we observe dephosphorylation of p107 as soon as 15 minutes post-treatment, data not shown), lysed, and immunoprecipitated with p107 antibodies. We

detected a marked increase in the association of the three subunits of the PP2A/B55α holoenzyme with p107 without changes in the expression of p107 or the subunits of the B55α/PP2A holoenzyme (Fig. 2B). This upregulation is transient, as it is not maintained at longer time points (data not shown). These data suggest that FGF1 induces formation of a PP2A/B55α-p107 complex via post-translational modification(s) on any of the subunits involved or the participation of an additional factor regulated by FGF1. We also determined the effects of FGF1 in primary rat chondrocytes obtained as described in the Materials and Methods section. Treatment of these cells with FGF1 promotes cell cycle arrest 24 h post stimulation (Fig. 2C). p107 dephosphorylation is clearly detected 2 h post stimulation, yet is not as complete as seen in RCS cells, likely due to the unavoidable heterogeneity of the primary cells (compare Fig. 2C, left panel to Fig. 1B). Immunoprecipitation of endogenous p107 from lysates of FGF1-treated rat chondrocytes with specific antibodies resulted in detection of a B55α/p107 complex, which peaked 2 h post-stimulation (Fig. 2D). Thus, the p107/B55α interaction is detectable in a rat cell line and in primary rat chondrocytes and it is inducible by FGF1.

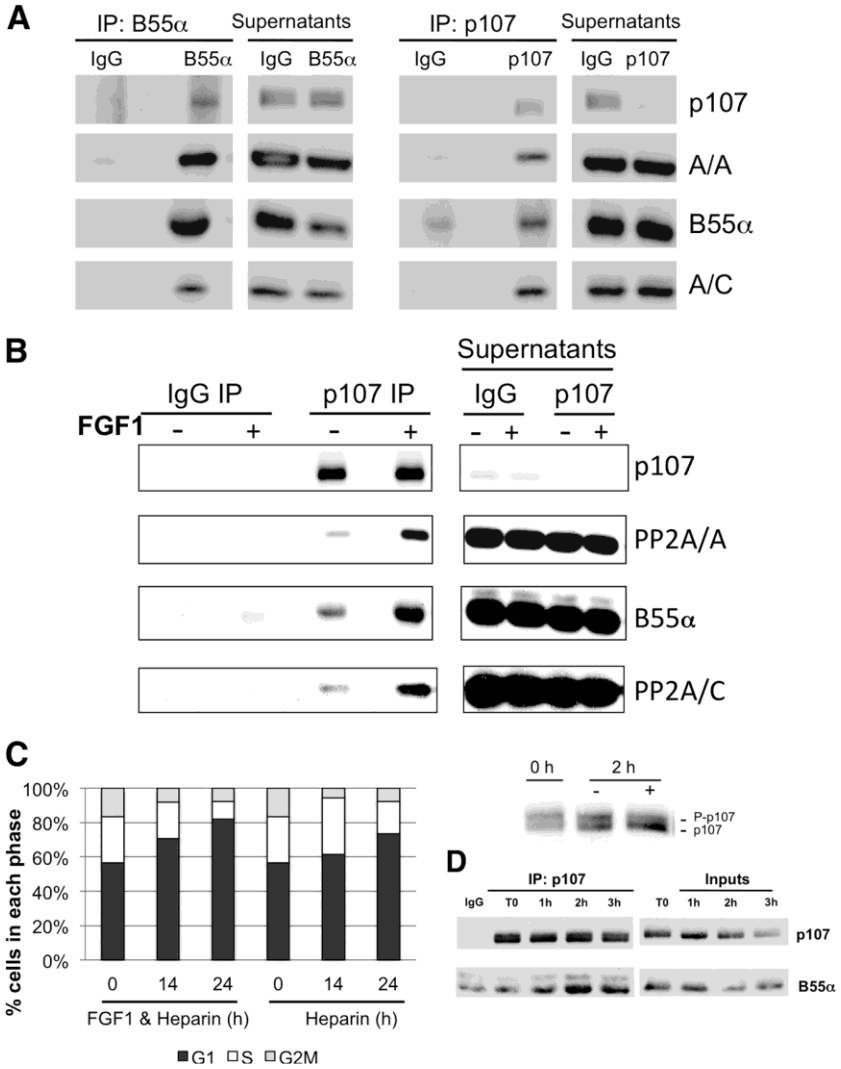

Figure 2. (A) Endogenous PP2A/B55α complexes co-immunoprecipitate with p107 in chondrocytes and the association is markedly increased upon FGF1 treatment in RCS

cells. Whole cell lysates were immunoprecipitated with the indicated antibodies and resolved by western blot analysis with antibodies to the indicated proteins (**A**, **B** and **C**). (**B**) FGF1 induces rapid transient formation of p107/B55α PP2A complexes coinciding with p107 dephosphorylation in RCS cells. RCS cells were stimulated with FGF1 in the presence of heparin for 30 min. (**C**) FGF1 induces p107 dephosphorylation and growth arrest in primary rat chondrocytes. Primary rat chondrocytes were treated with FGF1 (20 ng/ml) with heparin or heparin alone and cells were processed as in Fig. 1A (left panel) or for western blot analysis (right panel). (D) B55α co-immunoprecipitates with p107 in primary rat chondrocytes (Kurimchak et al, 2013).

To determine if B55α was the primary B regulatory subunit targeting the PP2A/A-C dimer to p107, we generated RCS cell lines ectopically expressing Myc-tagged B55α and Flag-tagged p107. Myc-B55α was expressed at about 75% the level of the endogenous protein (Fig. 3A, clone 3), while Flag-p107 was expressed at around ~120% the level of endogenous p107, and its phosphorylation state is modulated by FGF1 (Fig. 3B, clone 6). Both cell lines grew efficiently and maintained the transgene stably. We next performed immunoprecipitations from lysates of about 2.25 x 10^7 cells using antibodies against each tag. Immunoprecipitates were extensively washed, and tryptic peptides were subjected to LC-MS/MS analysis. As shown in Fig. 3C, the Flag-p107 proteomics analysis yielded peptides that allowed identification of multiple known partners of p107, including E2F/DP1 transcription factors, Cyclins and CDKs, multiple subunits of the DREAM complex (LIN proteins)(12), as well as a number of peptides corresponding to proteins not previously found as partners of p107 (Mybbp1a, p400 and Herc2). Importantly, we detected peptides corresponding to the catalytic, scaffold, and two members of the B family of regulatory PP2A subunits (B55α and B55δ). Based on the number of spectral hits, B55α is the preferred B subunit partner of p107, with B55δ being significantly less abundant (one peptide unique to B55δ, and two peptides common to B55α and B55δ). Of note, no other PP2A/B subunits nor subunits of any other phosphatase were detected in the proteomic analysis, suggesting a major role of B55α in modulation of p107 in chondrocytes. In striking agreement with these results, Fig. 3D shows that one of the major partners of the PP2A/B55α holoenzyme in these cells is p107, which was ranked as the 4th most abundant partner of B55α other than PP2A subunits by number of spectral hits. Interestingly, we also detected several peptides corresponding to pRB, suggesting that pRB is also a substrate of this holoenzyme (see discussion). To confirm these results, we performed immunoprecipitations using Flag-p107 cells and were able to verify many of the binding partners with more spectral hits, including Cyclins A and E and CDK2 and CDK4 (Fig. 3E) and PP2A subunits (not shown). Due to the high sensitivity of the LC-MS/MS and the unavailability of adequate antibodies for detection of endogenous interactions, some of the minor partners have yet to be confirmed via immunoprecipitation followed by western blot analysis.

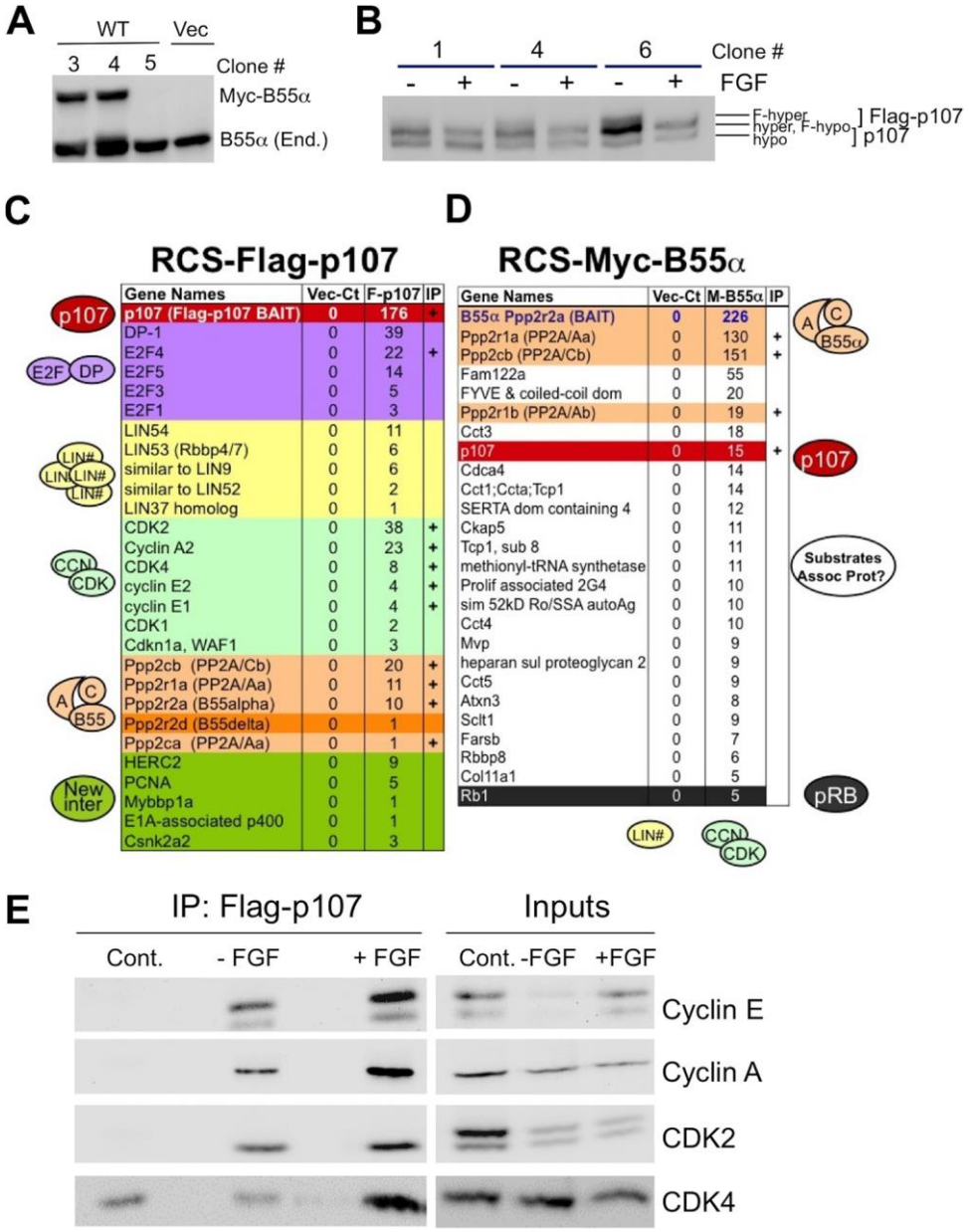

Figure 3. Generation of stable RCS-Flag-p107 and RCS-Myc-B55α cell lines and analysis of Myc-B55α and Flag-p107 complexes in RCS cells via solution-based proteomics analysis. Stable RCS-Myc-B55α and RCS-Flag-p107 clones were generated as described in the Materials and Methods section **(A and B)**. **(A)** RCS-Myc-B55α Clone 3 was selected for proteomics analysis. **(B)** Clone 6 was selected for proteomics analysis. Note that the hypophosphorylated form of Flag-p107 comigrates with endogenous hyperphosphorylated p107 (differentially phosphorylated forms are indicated). **(C** and **D)** RCS-Flag-p107, RCS-Myc-B55α and control RCS-vector cells were lysed and p107 and B55α complexes were immunoprecipated with corresponding anti-tag antibodies and processed for proteomics analysis as described in the Materials and Methods section. Numbers indicate the number of spectral hits and are ranked. (**C)** Proteins are grouped based on function as E2F/DP, LIN members of DREAM complex, Cyclin/CDKs, B55-PP2A

holoenzymes. The last group (green) contains potential unvalidated novel interactions. (**D)** B55α holoenzyme and potential substrates are indicated on the right. A few spectral hits were also detected for two LIN proteins, a cyclin and a CDK and are indicated on the right but not shown in this truncated table. If confirmed, these proteins could be associated with p107 or forming independent complexes with B55α. + *indicates confirmed by IP/WB analysis.* **(E)** Confirmation of select binding partners from proteomics analysis. RCS Flag-p107 cells were treated with FGF1 for 2 h, collected, immunoprecipiated using the M2 Flag antibody and subjected to WB analysis. Control indicates the parental RCS line (Kurimchak et al, 2013).

Aim 3. To determine if B55α is required for FGFα-induced dephosphorylation of p107 in chondrocytes.

We previously demonstrated that limited ectopic expression of B55α in U2-OS cells results in accumulation of hypophosphorylated p107, and this is not observed with a D197K point mutant that fails to bind p107 (Aim 2, below)(13). To determine whether limited expression of B55α in RCS cells induces p107 dephosphorylation, RCS cells were transfected with Myc-B55α-WT, Myc-B55α-D197K or an empty vector. Fig. 4A shows that limited expression of B55α-WT results in marked accumulation of hypophosphorylated p107, which does not occur in the cells expressing the empty vector or the Myc-B55α-D197K mutant. Conversely, cells transfected with two different B55α siRNAs exhibit increased levels of hyperphosphorylated p107 relative to the untransfected and scramble siRNA controls (Fig. 4B), demonstrating that relative small changes in the expression of B55α protein markedly affect p107 phosphorylation in RCS cells. To determine whether B55α is important for FGF1 mediated dephosphorylation of p107, we used two different shRNAs alone or in combination to knockdown B55α and subsequently treated the virally transduced cells with FGF1 for 30 minutes or 2 hours. We have noticed that shRNA knockdown of B55α in these cells is not very efficient. Nevertheless, knockdown of B55α by ~50% markedly delays FGF1-induced p107 dephosphorylation (Fig. 4C). At 30 minutes, p107 dephosphorylation is mostly complete in the FGF1 treated shRNA vector control cells, but not in the cells with B55α knockdown. This delay is still clearly detected by 2 hours of FGF1 treatment. Subsequently we used multiple siRNAs and double reverse transfection that resulted in more effective knockdown of B55α and delayed, but not completely blocked, FGF1-induced dephosphorylation (Fig 4D and 4E). It is possible that complete and/or more sustained knockdown of B55α is required to fully block FGF1 induced dephosphorylation of p107. However, since B55δ is likely a minor partner of p107 (Fig. 3C), it is also conceivable that B55δ compensates in a setting where B55α expression is reduced. Fig 4D shows that although B55δ siRNAs had little effect on p107 dephosphorylation, B55α/B55δ double knockdowns were more effective at delaying p107 dephosphorylation than B55α knockdown. Moreover, we also determined if the delays in FGF1-dependent p107 dephosphorylation resulting from siRNAs targeting either the ORF or the UTR of B55α could be attenuated by limited expression of a B55α transgene lacking its natural UTR. As expected (Fig. 4E, lower panel), expression of exogenous B55α resulted in more effective dephosphorylation of p107 and effectively prevented a dephosphorylation delay by the siRNAs targeting the UTR, which is clearly seen in the upper panel. However, the siRNA targeting the ORF could still prevent complete dephosphorylation of p107. Considering (i)

that multiple different shRNAs and siRNAs targeting B55α but not the vector/scramble controls promote p107 hyperphosphorylation and delay FGF1-dependent p107 dephosphorylation and (ii) the observation that FGF1 induces rapid formation of a B55α/PP2A holoenzyme complex with p107 coinciding with p107 dephosphorylation, we conclude that B55α is the major modulator of p107 phosphorylation state in RCS cells and the key upstream activator of p107 in FGF signaling in chondrocytes.

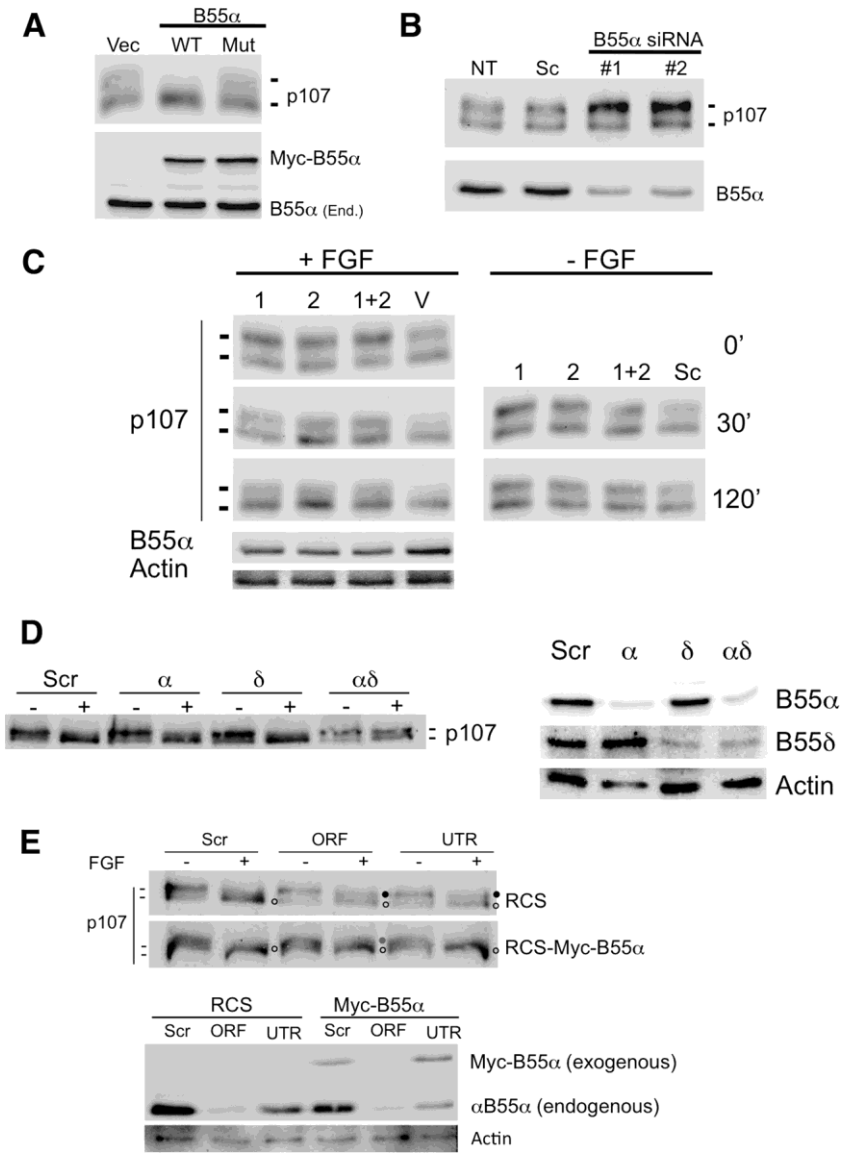

Figure 4. The phosphorylation state of p107 is sensitive to changes in the expression of B55α and loss of B55α function delays p107 dephosphorylation in response to FGF signaling RCS cells. (A) Overexpression of B55α results in a relative accumulation of hypophosphorylated p107, which is not observed with a D197K point mutant. (**B**) siRNA-mediated knockdown of B55α results in a relative increase of hyperphosphorylated p107 not seen in cells transfected with scrambled siRNA. **(C)** RCS cells were transduced with

lentiviruses encoding B55α or control shRNA vectors, selected with puromycin for three days and then stimulated with heparin alone (-FGF) or FGF and heparin (+FGF) for the times indicated. Proteins were detected via WB analysis. **(D)** siRNA-mediated knockdown of B55α and B55δ cooperate to prevent FGF1-induced p107 dephosphorylation (right panel). Levels of B55α and B55δ are shown on the right. **(E)** Expression of B55α resistant to siB55α directed to the UTR promotes p107 dephosphorylation even in the presence of B55α UTR siRNA. P107 hypophosphorylated (°) and hyperphosphorylated (•) forms are indicated (Kurimchak et al, 2013).

The remainder of the manuscript is beyond the aims of the proposal and was completed later. A model from these data emerged and it is show in Fig. 5 and summarized next. The data reported in the paper support a model in which FGF1 promotes assembly of PP2A/B55α holoenzymes with p107 by a mechanism that does not involve up-regulation of B55α protein levels (Fig. 5). The relative increase in PP2A/B55α holoenzymes targeting p107 shifts the equilibrium with CDKs towards rapid and selective dephosphorylation and activation of p107 without affecting pRB and p130, which remain hyperphosphorylated. Dephosphorylation of p107 results in its transient accumulation in the nucleus, remodeling of complexes including an increase in p107/E2F4 and p107/cyclin/CDK complexes, and p107 targeting to the *MYC* promoter (Figs. 5 and 6 in Kurimchak et al., 2013), which is downregulated early in this process as well as potentially other cell cycle and/or maturation genes. However, knockdown of B55α and/or B55∂ was not sufficient to prevent FGF1-mediated cell cycle exit although it led to a significant delay (Fig. 7 in Kurimchack et al., 2013). Because p130 and pRB remain hyperphosphorylated early on, and thus inactive, they do not seem to play an initiating role in controlling gene expression during the very early steps of chondrocyte maturation that culminate in cell cycle exit. Hours post-activation of p107, the CDK inhibitors p16 and p21 are upregulated (6,9), and in the case of p21 this coincides with Cyclin E/CDK2 inactivation and pRB dephosphorylation (6), which occurs in parallel with p130 dephosphorylation. This two-tier, sequential activation of pocket proteins and the demonstrated requirement of p107/p130 for chondrocyte cell cycle exit and endochondral bone formation (14,15) is likely in place to coordinate repression of cell cycle genes and perhaps induction of maturation genes (Fig. 5).

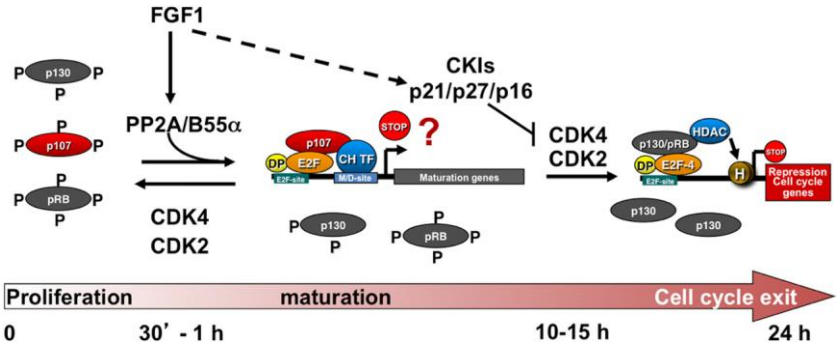

Figure 5. Differential effects of FGF1 on pocket proteins are mediated by the B55α-PP2A holoenzyme. In this model, FGF1 activates B55α PP2A-mediated dephosphorylation of p107 leading to rapid formation of p107 complexes that target genes regulated early in this

process. p130 and pRB remain at least partially hyperphosphorylated and do not play an initial role in controlling gene expression. FGF-dependent upregulation of p21, which results in inactivation of CDK2, and p16, which presumably inactivates CDK4 are likely responsible for the delayed dephosphorylation of these two pocket proteins. When p130 is activated, it may start substituting for p107 in repression of E2F-dependent genes, and/or may target new sets of genes. p107 typically forms repressor complexes with E2Fs, but other possibilities including positive transcriptional regulation with fate specific transcription factors are conceivably possible (Kurimchak et al, 2013).

Aim 2. To identify domains in B55α critical for binding to p107. The results of this aim were completed early after submission of the grant application because they were requested by reviewers of a manuscript that we had under review in JBC. Alison completed these experiments and the manuscript was accepted (Jayadeva, et al., 2010).

B55α consists of seven WD40 domains that form the blades of a β-propeller and a β-hairpin arm (Fig. 6A). p107 interacted both with the amino-terminal 1-216 amino acid fragment that contains the first three WD40 domains and the 217-447 C-terminus fragment that contains the last four WD40 domains in cotransfection assays in U-2 OS cells (data not shown). However, these deletion forms of B55α poorly interacted with PP2A/C and PP2A/A (Fig. 6A and data not shown). It has been recently reported that β-catenin, a substrate of B55α, also interacts with both domains (16). These results suggest that B55α substrates may make multiple contacts with the β-propeller structure. We also generated a series of point mutants targeting amino acids in the acidic top face of the β-propeller that have been previously found to be important for dephosphorylation of Tau by B55α (17). Fig. 6B shows that only residue D197 was essential for binding to p107 in reciprocal immunoprecipitation assays from lysates of transfected cells with the indicated constructs. This residue was also important for binding of B55α to PP2A/A and PP2A/C. Therefore, D197 is critical for p107 recruitment and efficient formation of the holoenzyme. It also appeared that substitution of residue K48 reduced binding to p107 despite efficient binding to PP2A/A and PP2A/C (Fig. 6B). Because mutations on B55α that affect dephosphorylation of Tau by the PP2A holoenzyme do not affect p107 binding, it is tempting to speculate that specific residues selectively distinguish between different substrates. Moreover, the identification of D197 as critical for binding of B55α and p107, provided as with a great control for gain of function studies.

In addition, this preliminary structural analysis became the seed for current studies were we are identifying the critical residues that determine substrate specificity using the same assay shown if Fig. 6B and a large collection of new B55α point mutants that more exhaustively scan the top acidic domain of the propeller.

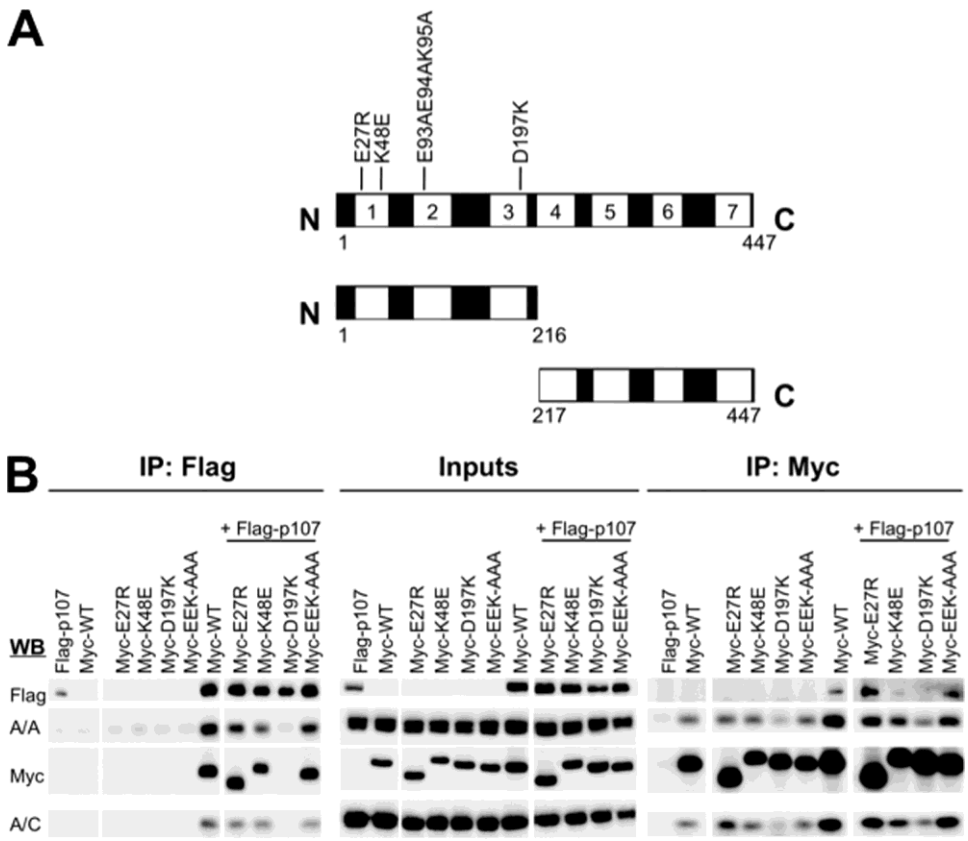

Fig. 6. B55α domains and residues required for binding to p107. (A) Structure of B55α and residues in its top acidic face that are important for dephosphorylation of the B55α substrate Tau. **(B)** B55α D197 is essential for the binding of p107. U-2 OS cells were co-transfected with Flag-p107 and the indicated Myc-B55α constructs. Cell lysates were immunoprecipitated with α-Flag and α-Myc antibodies and resolved via SDS-PAGE. Proteins were detected using α-Flag, α-Myc, α-PP2A/A and α-PP2A/C antibodies (Jayadeva, et al., 2010).

References

1. Kurimchak, A., and Graña, X. (2012) PP2A Counterbalances Phosphorylation of pRB and Mitotic Proteins by Multiple CDKs: Potential Implications for PP2A Disruption in Cancer. *Genes Cancer* **3**, 739-748
2. Kurimchak, A., and Graña, X. (2012) PP2A holoenzymes negatively and positively regulate cell cycle progression by dephosphorylating pocket proteins and multiple CDK substrates. *Gene* **499**, 1-7
3. Kurimchak, A., Haines, D. S., Garriga, J., Wu, S., De Luca, F., Sweredoski, M. J., Deshaies, R. J., Hess, S., and Graña, X. (2013) Activation of p107 by FGF, which is essential for chondrocyte cell cycle exit, is mediated by the PP2A/B55α holoenzyme. *Mol Cell Biol*

4. Sahni, M., Ambrosetti, D. C., Mansukhani, A., Gertner, R., Levy, D., and Basilico, C. (1999) FGF signaling inhibits chondrocyte proliferation and regulates bone development through the STAT-1 pathway. *Genes Dev* **13**, 1361-1366
5. Krejci, P., Bryja, V., Pachernik, J., Hampl, A., Pogue, R., Mekikian, P., and Wilcox, W. R. (2004) FGF2 inhibits proliferation and alters the cartilage-like phenotype of RCS cells. *Exp Cell Res* **297**, 152-164
6. Aikawa, T., Segre, G. V., and Lee, K. (2001) Fibroblast growth factor inhibits chondrocytic growth through induction of p21 and subsequent inactivation of cyclin E-Cdk2. *J Biol Chem* **276**, 29347-29352
7. Rozenblatt-Rosen, O., Mosonego-Ornan, E., Sadot, E., Madar-Shapiro, L., Sheinin, Y., Ginsberg, D., and Yayon, A. (2002) Induction of chondrocyte growth arrest by FGF: transcriptional and cytoskeletal alterations. *J Cell Sci* **115**, 553-562
8. Tran, T., Kolupaeva, V., and Basilico, C. (2010) FGF inhibits the activity of the cyclin B1/CDK1 kinase to induce a transient G_2 arrest in RCS chondrocytes. *Cell Cycle* **9**, 4379-4386
9. Dailey, L., Laplantine, E., Priore, R., and Basilico, C. (2003) A network of transcriptional and signaling events is activated by FGF to induce chondrocyte growth arrest and differentiation. *J Cell Biol* **161**, 1053-1066
10. Kolupaeva, V., Laplantine, E., and Basilico, C. (2008) PP2A-mediated dephosphorylation of p107 plays a critical role in chondrocyte cell cycle arrest by FGF. *PLoS One* **3**, e3447
11. Li, S., Brignole, C., Marcellus, R., Thirlwell, S., Binda, O., McQuoid, M. J., Ashby, D., Chan, H., Zhang, Z., Miron, M. J., Pallas, D. C., and Branton, P. E. (2009) The adenovirus E4orf4 protein induces G2/M arrest and cell death by blocking protein phosphatase 2A activity regulated by the B55 subunit. *J Virol* **83**, 8340-8352
12. Litovchick, L., Sadasivam, S., Florens, L., Zhu, X., Swanson, S. K., Velmurugan, S., Chen, R., Washburn, M. P., Liu, X. S., and DeCaprio, J. A. (2007) Evolutionarily conserved multisubunit RBL2/p130 and E2F4 protein complex represses human cell cycle-dependent genes in quiescence. *Mol Cell* **26**, 539-551
13. Jayadeva, G., Kurimchak, A., Garriga, J., Sotillo, E., Davis, A. J., Haines, D. S., Mumby, M., and Gra–a, X. (2010) B55alpha PP2A holoenzymes modulate the phosphorylation status of the retinoblastoma-related protein p107 and its activation. *J Biol Chem* **285**, 29863-29873
14. Cobrinik, D., Lee, M. H., Hannon, G., Mulligan, G., Bronson, R. T., Dyson, N., Harlow, E., Beach, D., Weinberg, R. A., and Jacks, T. (1996) Shared role of the pRb-related p130 and p107 proteins in limb development. *Genes & Development* **10**, 1633-1644
15. Laplantine, E., Rossi, F., Sahni, M., Basilico, C., and Cobrinik, D. (2002) FGF signaling targets the pRb-related p107 and p130 proteins to induce chondrocyte growth arrest. *J Cell Biol* **158**, 741-750
16. Zhang, W., Yang, J., Liu, Y., Chen, X., Yu, T., Jia, J., and Liu, C. (2009) PR55 alpha, a regulatory subunit of PP2A, specifically regulates PP2A-mediated beta-catenin dephosphorylation. *J Biol Chem* **284**, 22649-22656
17. Xu, Y., Chen, Y., Zhang, P., Jeffrey, P. D., and Shi, Y. (2008) Structure of a protein phosphatase 2A holoenzyme: insights into B55-mediated Tau dephosphorylation. *Mol Cell* **31**, 873-885

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
__X__ 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, and an abbreviated title of the

publication. For example, if you submit two publications for Smith (PI for Project 01), one publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

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. B55α PP2A Holoenzymes Modulate the Phosphorylation Status of the Retinoblastoma-related Protein p107 and Its Activation.	Jayadeva, G., Kurimchak, A,. Garriga, J., Sotillo, E., Davis, A., Haines, D. S., Mumby, M., and Graña, X.	**J. Biol. Chem.** 285: 29863-73. PMID: 20663872	July 2010	☐Submitted ☐Accepted ☒Published http://www.jbc.org/content/285/39/29863.long
2. Activation of p107 by FGF, which is essential for chondrocyte cell cycle exit, is mediated by the PP2A/B55α holoenzyme	Kurimchak, A; Haines, D; Garriga, J; Wu, S; Deluca, F.; Sweredoski; Deshaies, RJ; Hess, S. and Graña, X.	**Mol. Cell. Biol.** (16):3330-42).	Jan 2013	☐Submitted ☐Accepted ☒Published http://mcb.asm.org/content/early/2013/06/11/MCB.00082-13.full.pdf+html
3. PP2A holoenzymes negatively and positively regulate cell cycle progression by dephosphorylating	Kurimchak, A. & Graña, X.	**Gene** 499, 1-7. Invited Peer-reviewed review	December 2011	☐Submitted ☐Accepted ☒Published http://www.sciencedirect.com/science/article/pi

pocket proteins and multiple CDK substrates.				i/S0378111912 00217X
4. PP2A counterbalances phosphorylation of pRB and mitotic proteins by multiple CDKs: Potential implications for PP2A disruption in cancer.	Kurimchak, A. & Graña, X.	**Genes and Cancer** 499, 1-7. Invited Peer-reviewed review	November 2012	☐Submitted ☐Accepted ☒Published http://gan.sagepub.com/content/early/2013/02/27/1947601912473479.full

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

Yes_____ No___X____

If yes, please describe your plans:

All the results associated with the completion of the three aims have been published. Obviously, these results have served as preliminary data for other currently active studies that will lead to additional publications.

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.

The discoveries associated with this grant did not result in new drugs or new approaches for prevention, diagnosis and treatment.

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?
Yes_____ No____

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?
Yes_____ No____
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? Yes_____ No____

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 **Xavier Graña, Ph.D.**	POSITION TITLE **Associate Professor of The Fels Institute for Cancer Research** **Associate Professor of Biochemistry**
eRA COMMONS USER NAME xgrana	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Barcelona School of Biology, Spain	B.Sc	1982-1987	Biology
University of Barcelona Sch of Medicine, Spain	Ph.D.	1987-1991	Mol. Biol. & Biochem.
Cold Spring Harbor Laboratory, NY	Postdoctoral	1991-1992	Cell cycle control in
Fels Institute, Temple University Sch of Medicine,	Postdoctoral	1992-1994	normal and cancer cells

A. Personal Statement

Dr. Xavier Grana is a cancer biologist who has worked in the field of cell cycle regulation and control of gene expression since the initiation of his postdoctoral training at Cold Spring Harbor Laboratory in 1991. His lab has been funded by multiple grants from the NIH and private foundations since 1995. Dr. Grana has authored more than 50 peer-review publications in excellent molecular biology and oncology journals. Dr. Grana has trained numerous graduate students and postdoctoral fellows and in the Director of the Cancer Biology and Genetics Cluster in the Biomedical Science Graduate Program at TUSM.

B. Positions and Honors:

Positions and Employment

1994-1996	Research Assistant Professor, Fels Institute for Cancer Research, TUSM. Philadelphia.
1996-2000	Assistant Professor of Fels Institute for Cancer Research, TUSM. Assistant Professor of Biochemistry
1999-2009	Co-Director of the Molecular Biology and Genetics Graduate Program
2000-2001	Associate Professor of Biochemistry, TUSM.
2000-Present	Associate Professor of Fels Institute for Cancer Research
2001-Present	Associate Professor of Biochemistry, Tenured. TUSM. Philadelphia.
2009-Present	Director of the Molecular Biology and Genetics Graduate Program
2012-Present	Director, Cluster in Cancer Biology and Genetics, Bioscience Graduate Program

Other Experience and Professional Memberships

1994-present	Ad hoc reviewer: *Oncogene, Proc. Nat!. Acad.* Sci. *USA, EMBO J, EMBO Rep., Mol. Cell. Bioi., J. Bioi. Chem, Can. Res., Virology, Gene, J Cell Biochem, Mol. Hum. Rep., J. Cell Phys., CMLS,* J. *Immunol., Hepatology, Mol. Carcinogenesis, Trends Pharmacol, Mol. Bioi. Rep., Gene* and *Future Viral.*
1996-1999	Member, American Cancer SocietyllInstitutional Research Grant Review Committee.
1997-1998	Managing Editor *for Frontiers in Bioscience*.
1998	Association for International Cancer Research, Ad hoc, peer reviewer.
1998,1999	John Sealy Memorial Endowment Fund for Biomedical Research, ad hoc reviewer. Ohio
1999,2001	Cancer Research Associates. Adhoc, peer reviewer
2000-2003	NIH Cell Development and Function 6/F05 study section, frequent, ad hoc reviewer. NIH/NCI
2000, 2001	R21 review panel "Molecular Targets Drug Discovery," member
2003-2007	NIH Cell Signaling & Dynamics study section (previously CDF 3), member.
2005-present	BMC Cell Division, Associate Editor
2010-present	Gene, Member of the Editorial Board

Honors

1989-1990	Fellowship from CIRIT (Interdep. Commission Res. Technol Improvement). Spain.
1991-1993	Fulbright/MEC (Ministry of Education and Science of Spain) postdoctoral fellowship.
1993-1994	Lady Tata Memorial Award for Research in Leukemia and Allied conditions.
1994-1995	Fellowship from CIRIT. Generalitat de Catalunya. Spain.
1996	Individual allocation from American Cancer Society. "Regulation of p130" PI.

1996-1997 Wendy Will Case Grant: "Regulation of p130 During the Cell Cycle and Quiescence" PI
1996-2002 NIH First Award (R29): "Regulation of p130 During the Cell Cycle and Quiescence" PI.
1998-2002 WW Smith Charitable Trust Grant: "T-type cyclin/CDK9 in HIV replication." PI.
2000-2005 NIH/NIAID Career Development Award (K02)

C.**Selected Peer-reviewed Publications:** (selected from 55 peer-reviewed publications).

1. Mayol. X., Garriga, J. and Grana, ~. (1995) Cell cycle-dependent phosphorylation of the retinoblastomarelated protein p130. **Oncogene** 11, 801-808. PMID: 7651744.
2. Mayol, X., Garriga, J. & Grana,~. (1996). G1 Cyclin/CDK-independent phosphorylation and accumulation of p130 during the transition from G1 to GO lead to its association with E2F-4. **Oncogene** 13, 237-246.
3. Parrerio, M., Garriga, J., Limon, A., Mayol, X., Beck, G.R.Jr., Moran, E. and Grana, ~. (2000) E1A blocks hyperphosphorylation of p130 and p107 without affecting the phosphorylation status of the retinoblastoma protein. **J. Virol.** 74, (7), 3166-3176. PMC111817.
4. Calbo, J.; Parrerio, M.; Sotillo, E.; Yong, T.; Mazo, A.; Garriga, J. and Grana, ~. (2002) G1 cyclin/CDK coordinated phosphorylation of endogenous pocket proteins differentially regulates their interactions with E2F4 and E2F1 and gene expression. **J. Biol. Chern**. 277, 50263-50274. PMID: 12401786.
5. Bhattacharya, S.; Garriga, J., Calbo, J., Yong, T.; Haines, D.S. and Grana,~. (2003) SKP2 associates with p130 and accelerates p130 ubiquitylation and degradation in human cells. **Oncogene** 22, 2443-51.
6. Garriga, J.; Bhattacharya, S.; Calbo, J.; Marshall RM.; Truongcao, M.; Haines, DS. and Grana, ~. (2003). CDK9 is constitutively expressed throughout the cell cycle and its steady-state expression is independent of SKP2. **Mol. Cell. Biol**. 23, 5165-5173. PMC165719.
7. Garriga, J.; Jayaraman, AL.; Limon, A.; Jayadeva, G.; Sotillo, E.; Patsialou, A.; Truongcao, M.; Wadzinski, BE. and Grana, ~. (2004) A dynamic equilibrium between kinases and PP2A modulates phosphorylation of pRB, p107 and p130. **Cell Cycle** 3,1320-1330. PMID: 15467457.
8. Marshall, R.; Salerno, D.; Garriga, J. and Grana, ~. (2005) Cyclin T1 expression is regulated by multiple signaling pathways and mechanisms during activation of human peripheral blood lymphocytes. **J. Irnrnunol**. 175: 6402-6411. PMID: 16272292 (Article highlighted in JI, 2005,175: 6251-6252.
9. Reddy, HKDL; Mettus, MV; Rane, SG; Grana, ~.; Litvin, J. and Reddy, EP (2005) Cdk4 expression is essential for Neu-induced breast tumorigenesis. **Cancer Res**. 65(22):10174-10178.
10. Carbone, C.; Grana, ~.; Reddy, EP and Haines, DS (2007). p21 loss cooperates with INK4 inactivation facilitating immortalization and Bcl-2 mediated anchorage independent growth of oncogene-transduced primary mouse fibroblasts. **Cancer Res**. 67(9):4130-7. PMID: 17483323.
11. Sotillo, E.; Garriga, J.; Kurimchak, A. and Grana,~. (2008) Cyclin E and SV40 small t antigen cooperate to bypass quiescence and contribute to transformation by activating cdk2 in human fibroblasts **J. Biol. Chern**. 283(17):11280-92. PMC2431056.
12. Sotillo, E.; Garriga, J.; Padgaonkar, A; Kurimchak, A.; Cook, JG. and Grana, ~. (2009) Coordinated Activation of the Origin Licensing Factor CDC6 and CDK2 in Resting Human Fibroblasts Expressing SV40 Small T Antigen and Cyclin **J. Biol. Chem**. 284,14126 -14135. PMC2682861.
13. Rivadeneira, DB.; Mayhew, CN.; Sotillo, E.; Grana,~. and Knudsen, ES. (2010) Proliferative suppression by *CDK4/6* inhibition: complex function of the RB-pathway in liver tissue and hepatoma cells. **Gastoenterology**. 138(5):1920-30. PMC2860048
14. Garriga, J.; Xie, H.; Obradovic, Z. & Grana,~. (2009) Selective control of gene expression by CDK9. **J Cell Physiol**. 2009 Sep 24;222(1):200-208. PMC2783197
15. Jayadeva, G., Kurimchak, A,. Garriga, J., Sotillo, E., Davis, A., Haines, D. S., Mumby, M., and Grana, ~. (2010). B55a PP2A Holoenzymes Modulate the Phosphorylation Status of the Retinoblastoma-related Protein p107 and Its Activation. **J. Biol. Chem**. 285: 29863-73. PMID: 20663872 http://www.jbc.org/content/285/39/29863.long
16. Keskin, H., Garriga, J., Georlette, D. & Grana,~. (2012) Complex effects of flavopiridol on the expression of primary response genes. **Cell Div**. 7. PMID: 22458775
17. Kurimchak, A; Haines, D; Garriga, J; Wu, S; Deluca, F.; Sweredoski; Deshaies, RJ; Hess, S. and Grana,~. (2013). Activation of p107 by FGF, which is essential for chondrocyte cell cycle exit, is mediated by the PP2A/B55a holoenzyme **Mol. Cell. Biol**. (16):3330-42. *http://mcb.asm.org/contentlearly/2013/06/11* IMCB.00082-13.full.pdf+html

Invited reviews on the topic (in chronological order)

1. Grana, ~., Garriga, J. and Mayol, X. (1998) Role of the retinoblastoma protein family, pRB, p107 and p130 in the negative control of cell growth. **Oncogene** 17, 3365-3384. PMID: 9916999
2. Grana, ~. (2008). Downregulation of the Phosphatase Nuclear Targeting Subunit (PNUTS) triggers pRB dephosphorylation and apoptosis in pRB positive tumor cell lines**. Can. Bioi and Ther**. 14 (7) 824-826. P
3. Kurimchak, A. & Grana, ~. (2012) PP2A holoenzymes negatively and positively regulate cell cycle progression by de phosphorylating pocket proteins and multiple CDK substrates. **Gene** 499,1-7.
4. Kurimchak, A. & Grana, ~. (2013) PP2A counterbalances phosphorylation of pRB and mitotic proteins by multiple

CDKs: Potential implications for PP2A disruption in cancer. **Genes and Cancer** 499,1-7.