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 15 - NIBP/NFκB Signaling in Neurogenesis

7. **Start and End Date of Research Project:** 9/20/2011 – 6/30/2013

8. **Name of Principal Investigator for the Research Project:** Wenhui Hu, MD, PhD

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:

   $ $38,257.71

   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
Li, Fang	Lab manager	20%	10,803.00
Zhang, Yonggang	Postdoc	5%	2,536.00
Wang, Hong	Visiting Scholar	40%	16,100.00

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
Hu, Wenhui	PI	5%

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:
NIH/NCRR, 1R21RR032123; $275,000 (direct), 8/1/2011-7/31/2013

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:
Role and mechanism of NIBP/NFκB signaling in neurogenesis	☒NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	9/18/2012	$600,000 (total cost)	$600,000
HIV viral proteins regulate early differentiation of neural stem cells	☒NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	5/7/2012 1/4/2013 resubmitted	$275,000 (Direct cost)	$0
Identification and eradication of HIV-1 latent reservoir in neural stem cells	☒NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)	1/7/2014	$1,250,000 (Direct cost)	pending

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 plan to submit another NIH-R01 on the role of NIBP/NFκB signaling in the enteric neurogenesis.

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

NIBP is a novel protein involved in NFκB signaling and trans-Golgi networking. This project identified the potential role of NIBP/NFκB signaling in brain neurogenesis. We predict that NIBP/NFκB signaling plays similar role in the development of enteric nervous system. We will seed for additional funding in the gastroenterology field to study the role of NIBP in enteric neurogenesis.

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				2
Female				
Unknown				
Total				**2**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				2
Total				**2**

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				2
Other				
Unknown				
Total				**2**

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

Yes_____X____ No_____

If yes, please list the name and degree of each researcher and his/her previous affiliation: Hong Wang, MD, PhD, was a visiting scholar recruited from The First Affiliated Hospital, Zhejiang Chinese Medical University, China

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 research generated preliminary data for further grant application and funding, which brought new investigators to my lab for better research.

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:

The preliminary data from this research project built up the foundation for international collaboration with Dr. Xianming Mo at Sichuan University Huaxi Hospital, China. Such collaboration won a US-China NIH-R01 grant funding.

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 agreement). 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.

Specific Aims:

Neurodevelopmental disorders and cognitive/mood dysfunction are major public health issues. Impairments in embryonic and adult neurogenesis are implicated in such diseases. The molecular mechanisms that control various stages of neurogenesis remain poorly understood. We have identified a novel protein NIBP that is required for NFκB activation and neuronal differentiation as well as trans-Golgi networking. NIBP has been also implicated in human mental retardation and autism. The goal of this project is to understand the underlying cause-effects and mechanisms of NIBP/NFκB signaling in neurodevelopmental disorders. We will test

the central hypothesis that NIBP acts as a novel proneuronal protein to guide neuronal lineage differentiation through NFκB signaling during neurogenesis.

Specific Aim I: To determine the cellular distribution and developmental regulation of NIBP expression in the brain by using multi-labeled immunohistochemistry, in situ hybridization and realtime RT-PCR.
Specific Aim II: Delineate the role of NIBP in regulating neuronal lineage differentiation of neural stem/progenitor cells (NSCs/NPCs) using gain- and loss-of-function approaches.
Specific Aim III: To generate the floxed NIBP transgenic mice, which can be crossbred with various cell or tissue-specific Cre mice and thus generate multiple sources for further studies.

Summary of Research

Aim I: To determine NIBP expression pattern during neural development:

This aim was fully achieved as planned.

I-a. Developmental expression of NIBP: NIBP was identified from adult brain cDNA library. Bioinformatics suggest that NIBP is widely expressed in the nervous system. This is supported by *in situ* hybridization mapping of mouse adult brain showing extensive expression of NIBP with highest levels in the hippocampus. Using our own NIBP polyclonal antibody, we performed immunohistochemical staining of mouse embryos at various stages of development. As shown in Fig. 1, NIBP expression was undetectable in neuroepithelial cells (E9), started in NSCs/NPCs (E10-12) and gradually increased in more mature neurons (E13-16). *These expression profiles suggest that NIBP may promote embryonic neurogenesis and neuronal maturation*.

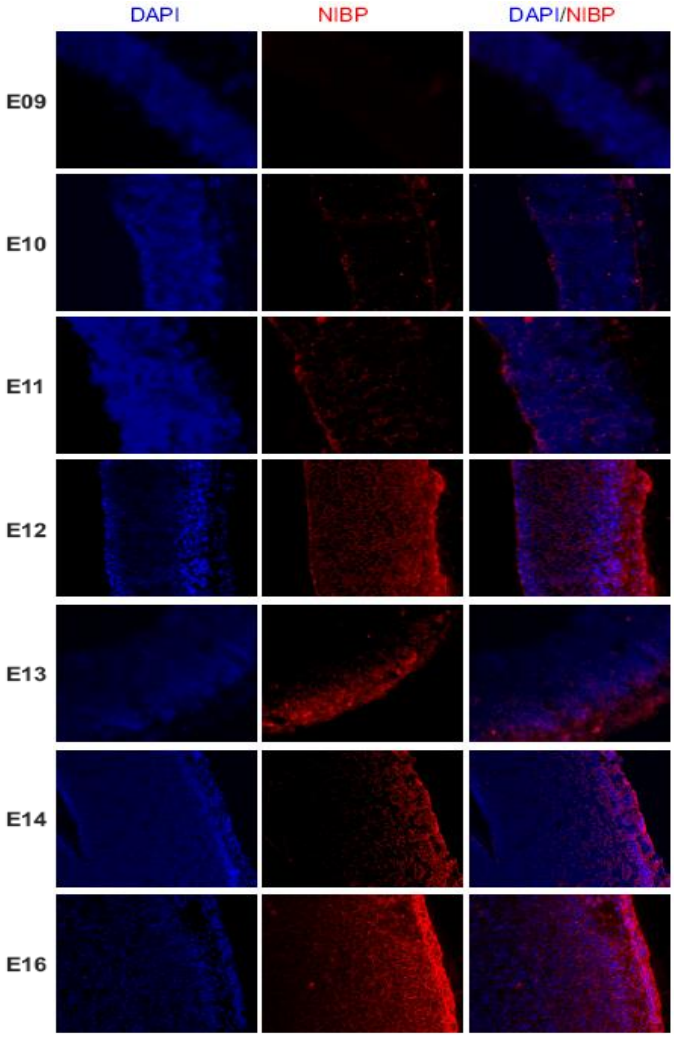


Fig.1. Immunohistochemical staining of mouse embryonic (E) cerebral cortex.

To further determine the expression pattern of NIBP during neural induction from embryonic stem (ES) cells, we established an *in vitro* model for neural induction and neuronal lineage differentiation. Mouse ES cells were cultured in neural induction media to generate embryoid bodies (EBs) following a well-established protocol. EBs were

then cultured in NSC proliferation medium to generate primitive neurospheres. After passage 2-3, the neurospheres were dissociated into single cells and underwent differentiation in NSC differentiation media. Representative pictures in Fig. 2A-D showed the successful neural induction from ES cells and neural lineage differentiation. To assess the expression levels of various NIBP transcripts during neural induction, quantitative RT-PCR analysis with several pairs of NIBP primers (Fig. 3A) was performed using total RNA harvested at each stage of neural induction. As shown in Fig. 3, NIBP mRNA expression was increased significantly in NSCs as compared with that in EBs and ES cells, which exhibited detectable levels of NIBP mRNA expression (Fig. 3B, C). These data support the above observation with NIBP antibody during embryonic neural development that NIBP starts expression at E9-10 in mouse brain.

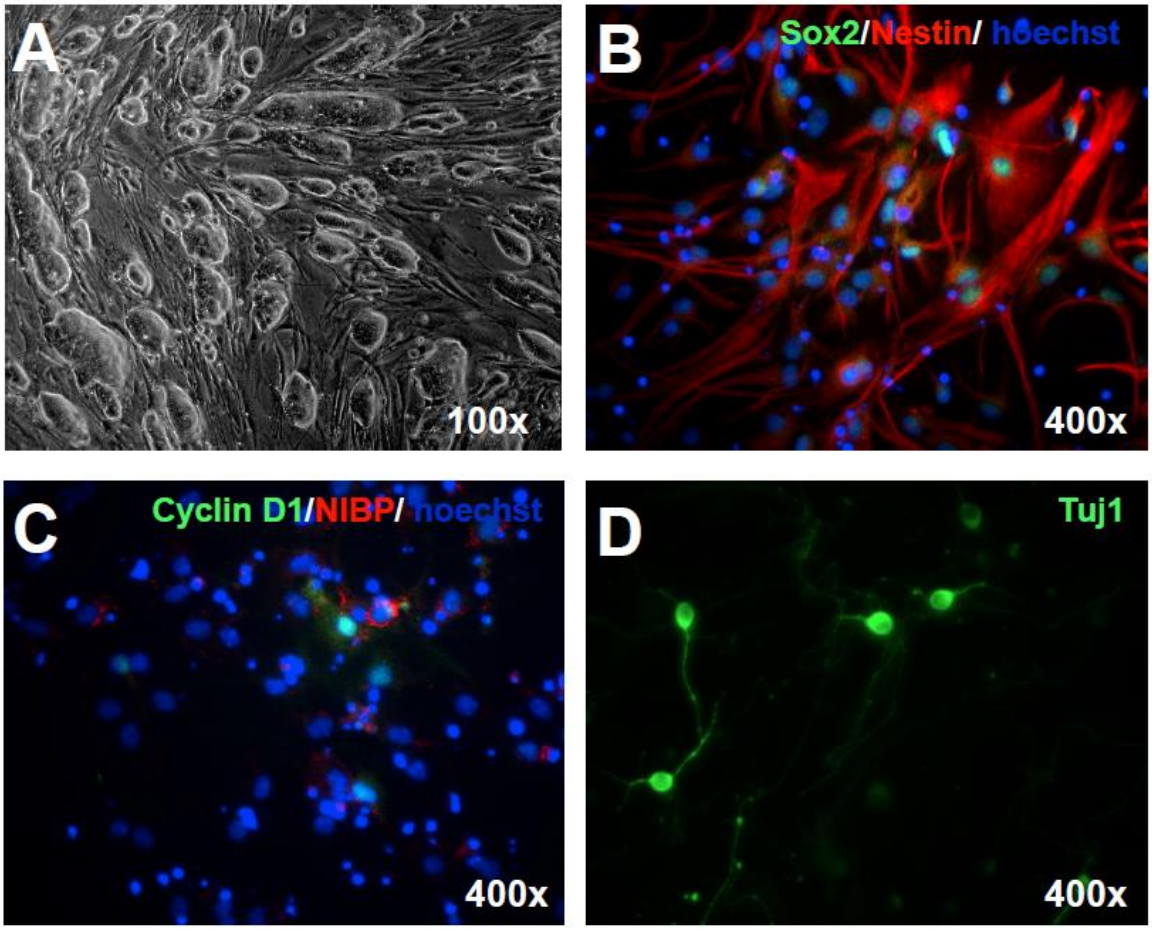


Fig. 2. Increased NIBP expression during neural induction of mouse ES cells. ***A***, typical ES cells. ***B, C***. NSCs labeled by Sox2/Nestin showed some NIBP-expressing cells. ***D.*** NSCs after 2 days' differentiation showed Tuj1-positive neurons.

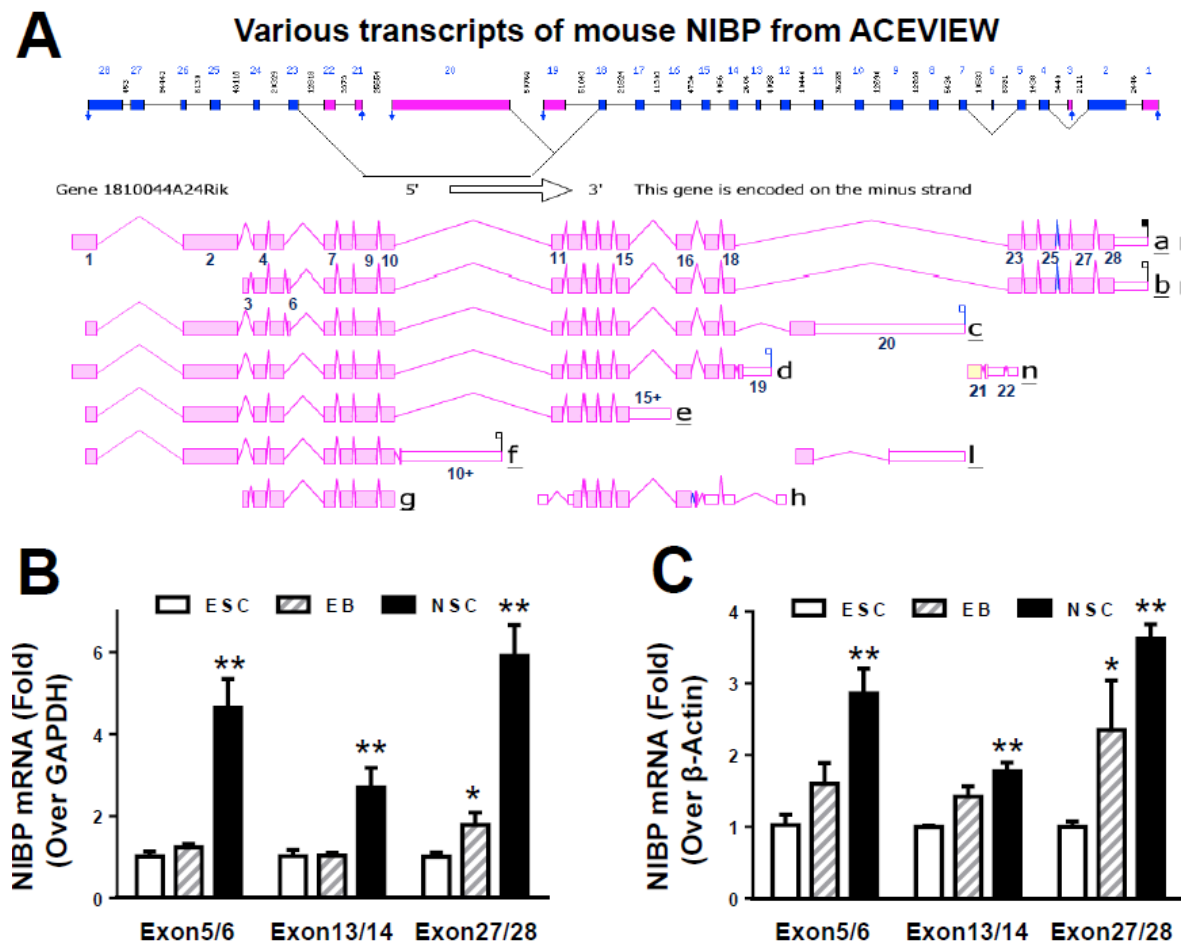


Fig. 3. Quantitative RT-PCR analysis showed significant high expression of NIBP transcripts in cultured NSCs. ***A.*** Various transcripts of NIBP identified by bioinformatics from Genebank. ***B, C.*** Different expression pattern of NIBP transcript variants during neural induction. *p<0.05 and **p<0.01 indicate significant changes relative to ESCs after normalization by GAPDH (B) or β-actin (C). More dramatic changes occurred to the N-terminal and C-terminal regions.

I-b. NIBP expression in neural stem cells (NSCs): In order to identify which isoform of NIBP is expressed in various stages of neurogenesis, we have generated a monoclonal antibody against the N-terminal domain of NIBP(1139), which recognizes the full-length of NIBP. As shown in Fig. 4, this antibody specifically immunoprecipitated (I.P.) endogenous NIBP in brain tissues and recognized GFAP-positive NSCs but not Tuj1-positive immature neurons in monolayer culture of SVZ NSCs/NPCs under proliferation

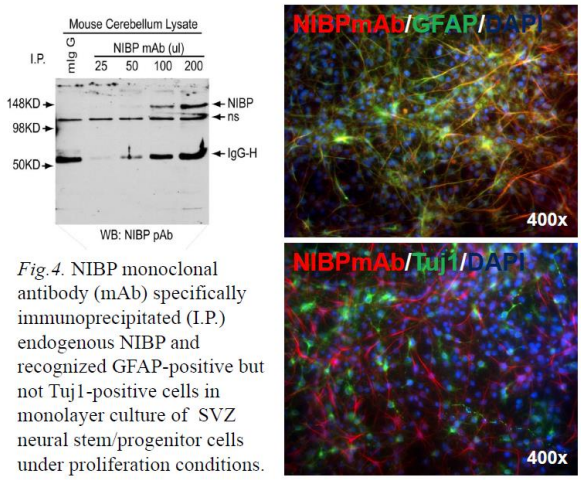


Fig.4. NIBP monoclonal antibody (mAb) specifically immunoprecipitated (I.P.) endogenous NIBP and recognized GFAP-positive but not Tuj1-positive cells in monolayer culture of SVZ neural stem/progenitor cells under proliferation conditions.

conditions.
Immunohistochemistry of neurogenic zone in adult brain using NIBP monoclonal and polyclonal antibodies showed that NIBP is expressed in NSCs/NPCs but not neuroblast (Fig. 5).

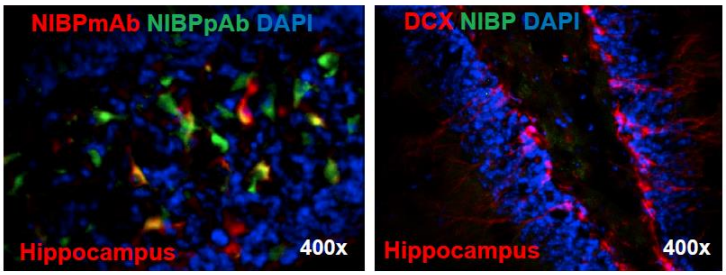


Fig.5. NIBP-like-immunoreactivity is expressed in neural stem/progenitor cells and mature neurons (left) but not neuroblast (right)

Aim II: To determine the role of NIBP in directing neuronal lineage differentiation:

This aim was fully achieved.

<u>II-a. Effects of NIBP knockdown or overexpression in cultured NSCs/NPCs:</u>
In cultured mouse subventricular zone (SVZ) NSCs, lentivirus-mediated stable NIBP siRNA knockdown inhibited neuronal differentiation whereas lentivirus-mediated NIBP overexpression promoted neuronal differentiation and inhibited astrocytic differentiation (Fig. 6, 7), suggesting that NIBP preferentially guides neuronal lineage differentiation.

To determine the potential role of NIBP in vivo in regulating neuronal lineage differentiation, we performed microinjection of lentivirus carrying

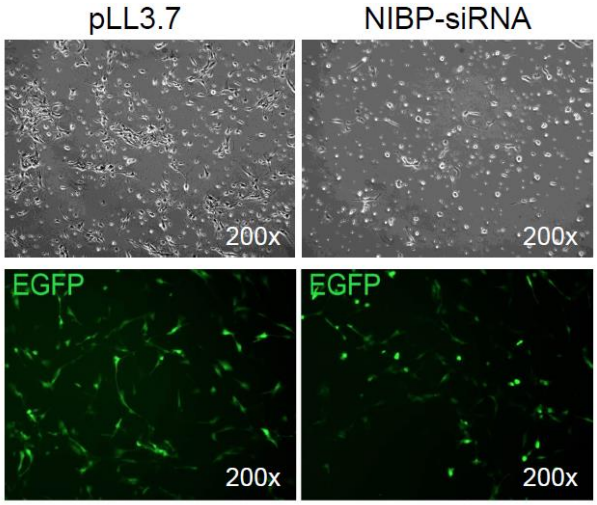
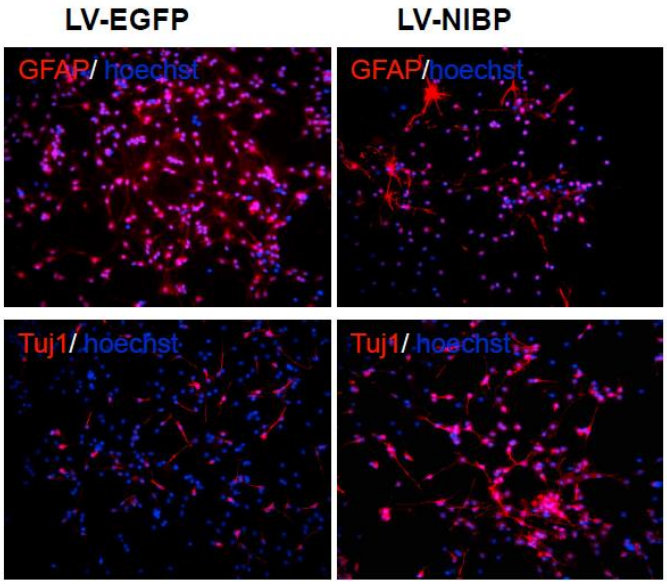


Fig.6A. **Lentivirus-mediated siRNA of NIBP inhibits differentiation in mouse adult neural stem cells.** Primary NSCs were infected with lentivirus carrying empty vector pLL3.7 or NIBP-siRNA for 4 h and cultured till formation of secondary neurospheres. Dissociated NSCs were plated on matrigel-coated plate and micrographs were taken at 12 h (*top*) or 7 d (*bottom*).



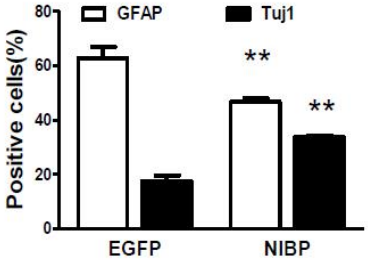


Fig.6B. **Lentivirus (LV)-mediated NIBP over-expression in adult NSCs promotes neuronal differentiation but inhibits astroglial differentiation.** Immunocytochemistry was performed at 3 days after differentiation. ** P<0.01 indicates significant difference from corresponding EGFP control

effective NIBP-shRNA into neonatal mouse SVZ. The empty shRNA lentiviral vector was used as a negative control. As shown in Fig. 8, stable knockdown of NIBP significantly inhibited the differentiation, migration and neuronal maturation of the transplanted NSCs during olfactory neurogenesis.

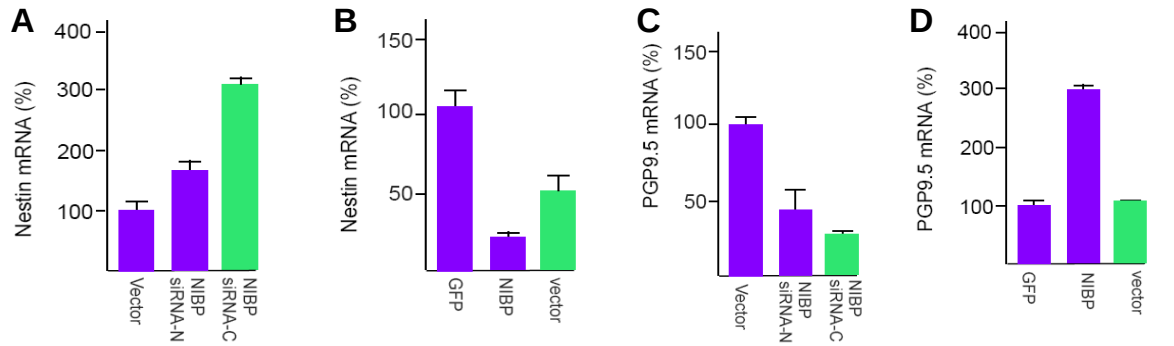


Fig.7. Quantitative RT-PCR analysis in neural stem cells showing that NIBP siRNA (A, C)) increased Nestin but decreased PGP9.5 expression. In contrast, NIBP overexpression (B, D) decreased Nestin but increased PGP9.5 expression. Primary neural stem cells were infected with lentivirus carrying NIBP-siRNA expression cassette or NIBP full-length expression vector. At 2 days postinfection, total RNA was extracted and SybrGreen RT-PCR was performed. Data represent mean ±SD (n=3-5)

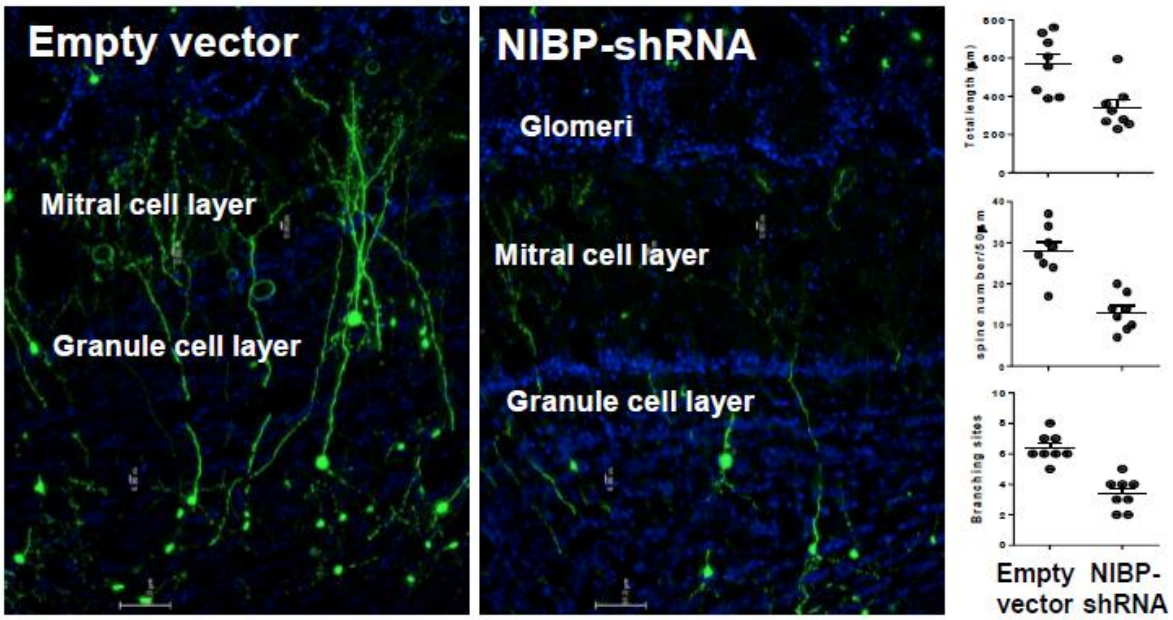


Fig.8. Lentivirus -mediated NIBP shRNA knockdown in neonatal SVZ NSCs/NPCs promotes neuronal differentiation and maturation. Lentivirus (5 µl, 1x10e5/µl) was injected into lateral ventricle of neonatal P1 mice. After 1 month, animals were perfused with 4% paraformaldehyde and frozen brain sections were evaluated for EGFP positive neurons and their dendrites in olfactory bulb.

II-b. NIBP knockout in cultured NSCs/NPCs: To determine whether NIBP knockout affects neuronal lineage differentiation, we cultured SVZ NSCs/NPCs from homozygote (f/f), heterozygote(+/f) and wild-type (+/+) of RCE/NIBP littermates (see Aim III) and treated the cells with 4-hydroxyl-tamoxifen (4-HT) to induce NIBP knockout in NSCs/NPCs. As shown in Fig. 9, Cre-mediated recombination efficiently deleted the designated fragment of NIBP gene (Fig. 9B). The resulting PCR product was validated by sequencing (Fig. 9C). By 4 days after induction, NIBP protein was undetectable (Fig. 9D). Neuronal differentiation and neurite branching were dramatically inhibited by NIBP knockout (Fig. 10). *These pilot studies support the feasibility to knock down or knock out the NIBP gene in NSCs/NPCs and demonstrate that NIBP is essential for neuronal lineage differentiation and neurite branching.*

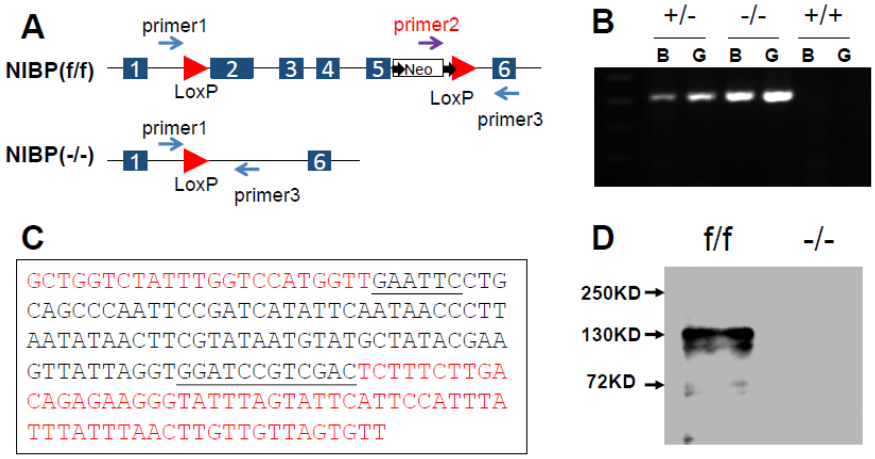


Fig. 9. Tamoxifen-induced Cre-mediated deletion of NIBP in neural stem cells. Neurospheres from brain (B) and gut (G) of RCE/NIBP floxed mice were treated with 4-hydroxyl tamoxifen (1 μM, 4h). After 48 h, genomic DNA was extracted for PCR genotyping with primer 1/3 (**A, B**). The PCR product was sequenced with primer 1, showing expected sequence after deletion of exon 2-5 (**C**). Underlined nucleotides represent introduced digestion sites (*EcoR*I and *BamH*I/*Sal*I). NIBP protein expression was detected by Western blotting with anti-NIBP antibody (**D**).

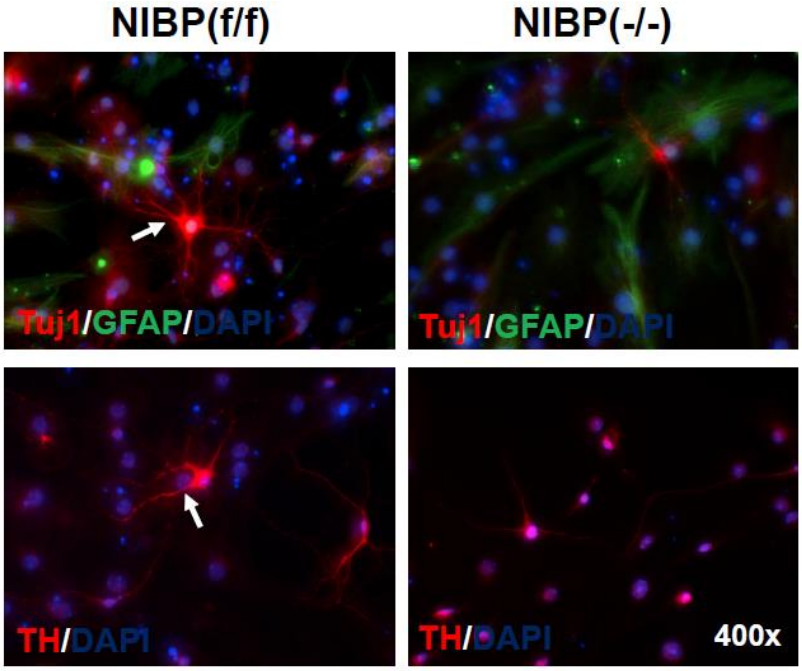


Fig. 10. NIBP deletion dramatically inhibits neuronal differentiation and neurite branching (Arrow) in neural stem cells. After differentiation for 4 days, immunocyto-chemistry was performed with antibodies against neuronal marker Tuj1 and tyrosine hydroxylase (TH), and astrocyte marker GFAP. Micrographs were taken under 40x objective.

Aim III: To develop floxed NIBP conditional transgenic mice:

This aim was fully achieved as planned.

III-a. Generation of floxed NIBP mice: Due to embryonic lethality of conventional NIBP knockout mice, we initiated the generation of floxed NIBP transgenic mice using Cre-loxP system. Because of unknown functional domains within NIBP, we selected a targeting strategy to remove a long fragment (8 kb) containing exon 2-5 using novel λ-Red recombineering technique. Southern blotting and PCR analysis confirmed homologous recombination for both arms of the targeting cassette. Cre-mediated deletion of floxed fragment in positive embryonic stem (ES) cells was verified by Adeno-Cre-EGFP transduction and PCR genotyping. Three (D1, E3 and H4) of 15 positive ES clones were selected for microinjection into C57BL/B6 blastocytes in Jefferson Transgenic Core (http://www.jci.tju.edu/transgenic/). One chimera for each D1 and E3 ES clone was obtained but no chimera for H4 was generated. Resulting chimeras from D1 and E3 ES clones crossed with B6 yielded heterozygous NIBP(+/f) mice, implying successful germline transmission for both lines. Intercrossing of NIBP(+/f) mice generated homozygous NIBP(f/f) mice with the expected Mendelian ratio. These two lines were crossbred with Flp transgenic mice to remove Frt-flanked neomycin gene. The heterozygous and homozygous mice of both genders for each line (D1 and E3) were healthy and fertile.

III-b. Universal and cell-specific NIBP knockout: We focused on D1 line for further genotyping and phenotyping and maintained E3 line for backup. Crossbreeding of Rosa-Cre-ERT(RCE) mice with NIBP(f/f) mice generated heterozygous double-transgenic RCE(+/-);NIBP(+/f) mice. Intercrossing of F1 offspring yielded homozygous RCE;NIBP(f/f) mice, which are healthy and fertile in both genders. The feasibility and efficiency of inducible NIBP knockout were validated by *in vitro* culture of NSCs/NPCs as shown above.

Abstract/poster presentation:

1. Zhang YG, Li F, Mo XM and Hu WH. NIBP/NFκB signaling regulates neuronal differentiation of enteric neural stem cells. Digestive Disease Week (DDW), Chicago, May 7, 2011.
2. Zhang YG, Li F, Mo XM and Hu WH. NIBP/NFκB signaling initiates early neuronal differentiation of neural stem cells through repressing C/EBPβ. Keystone Symposium: Neurogenesis (J7) and New Frontiers in Neurodegenerative Disease Research (J8). Santa Fe, February 3-8, 2013

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, 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.				☐Submitted ☐Accepted ☐Published
2.				☐Submitted ☐Accepted ☐Published
3.				☐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:

One publication is in the process of being written up

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?
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

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.

Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME Hu, Wen-Hui	POSITION TITLE Associate Professor
eRA COMMONS USER NAME wenhuihu	

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
North Sichuan Medical College, Sichuan, China	M.D.	1982	Medicine
Third Military Medical University, Chongqing, China	M.S.	1988	Traumatic Surgery
Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing, China	Ph.D.	1996	Molecular Neurobiology
National Jewish Medical and Research Center, University of Colorado, Denver, CO	Postdoc Fellow	1999-2000	Molecular Biology/ Immunology
Miami Project to Cure Paralysis, University of Miami, Miami, FL	Postdoc Associate	2000-2002	Neurosurgery/ Neuroimmunology

A. Personal Statement

I am a physician scientist and have over 28 years of experience in molecular/cellular neuroscience, with specific training and expertise in gene regulation (NIBP and RGS4), signal transduction (NFκB and G protein signaling) and novel biotechnology (siRNA, Cas9, iPS). After college study, I had three years' experience in clinical works. Then I obtained my graduate training in traumatic surgery and molecular neurobiology, focusing on spinal cord injury and regenerative medicine. As a postdoctoral fellow at National Jewish Medical Center, I started my training on NFκB signaling and identified a novel TNF family member TALL-1. At the Miami Project to Cure Paralysis, I expanded my research in NFκB signaling and initiated studies on neuroregeneration inhibition and neuronal differentiation. Using yeast two-hybrid screening, I identified several novel proteins regulating NFκB signaling and nerve growth inhibition. NIBP was my most favorite protein and kept motivating me for science. I also generated GFAP-dnIκBα transgenic mice, which has led to several peer-reviewed high impact publications. As PI or co-Investigator on several NIH-funded grants in Virginia Commonwealth University (VCU), I continued working on NFκB signaling in enteric neuroscience. Here at Temple University (TU), I have run my own lab for over 5 years to accomplish my commitment to the current renewed NIH grant and initiated several new projects (see funding list by DOD, NIH-R21 and NIH-R01).

In summary, my strong research experience and productive record in transgenic animal modeling, neurogenesis and signal transduction, as well as my relevant administrative experience and fruitful collaboration have made me fully qualified to lead this project.

B. Positions and Honors

Positions and Employment

1982-1985 Medical Residency, Internal Medicine, 520 Hospital, National Committee for Science and Technology, Sichuan, China
1988-1993 Lecturer, Research Institute of Surgery and Daping Hospital, The Third Military Medical University, Chongqing, China
1996-1999 Associate Professor, Research Institute of Surgery and Daping Hospital, The Third Military Medical University, Chongqing, China
2002-2004 Assistant Scientist, Miami Project to Cure Paralysis, University of Miami, Miami, FL
2004-2008 Assistant Professor, Department of Physiology, Virginia Commonwealth University, Richmond, VA
2007- Visiting Associate and Full Professor, West China Hospital, Sichuan University, Chengdu, China
2008- Assistant Professor (Tenure-Track), Department of Neuroscience, Temple University, Philadelphia, PA

Other Experience and Professional Memberships

1999-2004 The American Society for Biochemistry and Molecular Biology (ASBMB)
2000- The Society for Neurosciences (SFN)
2004- The American Gastroenterological Association (AGA)
2009- The American Physiological Society (APS)
2009- The American Neurogastroenterology and Motility Society (ANMS)
2010- The International Society for NeuroVirology (ISNV)

Honors and Awards

1996 Dr. Kequan Ge Chinese American Scholarship in Medical Research, China
1998 Military Science and Technology Achievement Award, China
1999 Beijing Science and Technology Achievement Award, China
1999 Janel S. Lewald Fellowship in Basic Immunology, University of Colorado, Denver
2000 Lois Pope Postdoctoral Fellowship in Neuroscience, University of Miami, Miami
2000 Military Science and Technology Achievement Award, China
2005 Military Science and Technology Achievement Award, China

C. Selected peer-reviewed publications (From 58 articles peer-reviewed publications)
Most relevant to the current application (in chronological order)

1. **Hu WH,** Mo XM, Walters WM, Brambilla R, and Bethea JR. TNAP, a novel repressor of NFκB-inducing kinase, suppresses NFκB activation. J Biol Chem 2004; 279(34):35975-35983.
2. **Hu WH**, Pendergast JS, Mo XM, Brambilla R, Bracchi-richard V, Li F, Walters WM, Blits B, He L, Schaal SM and Bethea JR. NIBP: A Novel NIK and IKKβ binding protein that enhances NFκB activation, J Biol Chem 2005; 280: 29233-29241.

3. Brambilla R, Bracchi-richard V, **Hu WH,** Bramwell A, Karnally S, Green EJ and Bethea JR. Inhibition of astroglial NFκB reduces inflammation and improves functional recovery following spinal cord injury. J Exp Med 2005; 202: 145-156.
4. Bracchi-richard V, Brambilla R, Levenson J, **Hu WH**, Bramwell A, Sweatt JD, Green EJ and Bethea JR. Astroglial NFκB regulates learning and memory and synaptic plasticity in female mice. J Neurochem 2007, 104(3):611-623.
5. Sun D, Gugliotta M, Rolfe A, Reid W, McQuiston AR, **Hu WH**, Young H. Sustained survival and maturation of adult neural stem/progenitor cells after transplantation into the injured brain. J Neurotrauma. 2011; 28(6):961-72
6. Zhang Y, Liu J, Yao S, Li F, Xin L, Lai M, Bracchi-Richard V, Xu H, Yen W, Meng W, Liu S, Yang L, Karmally S, Liu J, Zhu H, Gordon J, Khalili K, Srinivasan S, Bethea JR, Mo X, **Hu WH**. NFκB signaling initiates early differentiation of neural stem cells. Stem Cells 2012;30(3):510-24
7. Zhang Y and **Hu WH**. NFκB signaling regulates embryonic and adult neurogenesis. Front Biol 2012; DOI 10.1007/s11515-012-1233-z

<u>Additional recent publications of importance to the field(in chronological order)</u>

1. **Hu WH**, Johnson H and Shu HB. Tumor necrosis factor-related apoptosis-inducing ligand receptors signal NFκB and JNK activation and apoptosis through distinct pathways. J Biol Chem 1999; 274(43):30603-30610.
2. **Hu WH**, Johnson H and Shu HB. Activation of NFκB by FADD, casper, and caspase-8. J Biol Chem 2000; 275(15):10838-10844.
3. Hausmann ON, **Hu WH**, Keren-Raifman T, Witherow DS, Wang Q, Levay K, Frydel B, Z Slepak V, R Bethea JR. Spinal cord injury induces expression of RGS7 in microglia/macrophages in rats. Eur J Neurosci 2002; 15(4):602-612.
4. **Hu WH**, Walters WM and Bethea JR. Identification and characterization of a novel Nogo-interacting mitochondrial protein (NIMP). J Neurochem 2002; 81(1):35-46.
5. **Hu WH**, Walters WM, Xia XM, Karmally SA and Bethea JR. Neuronal glutamate transporter EAAT4 is expressed in astrocytes. Glia 2003, 44(1):12-25
6. **Hu WH,** Huang J, Mahavadi S, Li F and Murthy KS. Lentiviral siRNA silencing of sphingosine-1-phosphate receptors S1P1 and S1P2 in smooth muscle. Biochem Biophys Res Commun 2006; 343: 1038-1044
7. **Hu WH,** Mahavadi S, Huang J, Li F and Murthy KS. Characterization of S1P1 and S1P2 receptor function in smooth muscle by receptor silencing and receptor protection. Am J Physiol Gastrointest Liver Physiol 2006; 291(4):G605-10
8. Zhou HP, Jarujaron S, Gurley EC, Ding H, Chen L, Studer E, **Hu WH**, Pandak WM, Zou T, Wang JY and Helemon PB. HIV Protease Inhibitors Increase TNF-α and IL-6 Expression in Macrophages: Involvement of the RNA-Binding Protein HuR. Atherosclerosis 2007,195(1):e134-e143
9. **Hu WH**, Mahavadi S, Li F and Murthy KS. Upregulation of RGS4 and downregulation of CPI-17 mediate inhibition of colonic muscle contraction by IL-1β. Am J Phsyiol Cell Physiol 2007; 293(6):C1991-2000.
10. **Hu WH**, Li F, Mahavadi S and Murthy KS. Interleukin-1β up-regulates RGS4 through the canonical IKK2/IκBα/NFκB pathway in colonic smooth muscle. Biochem J 2008,412(1):35-43.

11. **Hu WH**, Li F, Mahavadi S and Murthy KS. Up-regulation of RGS4 expression by interleukin-1β in colonic smooth muscle is enhanced by ERK1/2 and p38 MAPK and inhibited by PI3K/Akt/GSK3β pathway. Am J Phsyiol Cell Physiol 2009 296(6):C1310-1320.
12. Li F, Murthy KS, Khalili K, **Hu WH**. Cloning and characterization of rabbit RGS4 promoter in gut smooth muscle. Gene. 2010; 451(1-2):45-53.
13. Li F, Hu DY, Liu S, Mahavadi S, Yen W, Murthy KS, Khalili K, **Hu WH**. The RNA-binding protein HuR regulates RGS4 mRNA stability in rabbit colonic smooth muscle cells. Am J Phsyiol Cell Physiol 2010; 299(6):C1418-29
14. Kuang X, **Hu WH**, Yan M, Wong PK. Phenylbutyric acid suppresses protein accumulation-mediated ER stress in retrovirus-infected astrocytes and delays onset of paralysis in infected mice. Neurochem Int. 2010; 57(7):738-48
15. Zhang Y, Li F, Liu S, Mahavadi S, Murthy KS, Khlili K, **Hu WH**. MEKK1-MKK4-JNK-AP1 pathway negatively regulates Rgs4 expression in colonic smooth muscle cells. PlosOne 2012; 7(4):e35646

Patents:

US Provisional Application No. 61/251,013 and the corresponding International Application No. PCT/US2010/052302, titled, 'Use of NIBP Polypeptides', published on April 21, 2011.

US Provisional Application No. 61871626, titled "RNA guided eradication of human HIV-1 and other retroviral genomes"

D. Research Support

1R01MH101041-01, Wenhui Hu (PI), 9/1/2013-8/31/2016
NIH/NIMH,
"Role and mechanism of NIBP/NFκB signaling in neurogenesis"
The major goal of this project is to characterize the role and mechanisms of NIBP/NFκB signaling in neurogenesis using mouse and zebrafish animal models.

R01, 2R01DK075964-06A1, Wenhui Hu (PI), 04/01/2007-8/31/2017
NIH/NIDDK
"Regulation of gastrointestinal neuromuscular function by NIBP/NFκB signaling"
The major goal of this project is to determine the effects of NIBP/NFκB signaling on the major signaling targets mediating intestinal smooth muscle contraction/relaxation.

Completed Research Support

1R21RR032123-01A1 Wenhui Hu, Ph.D. (PI) 8/1/2011-7/31/2013
NIH/NCRR
"Development and characterization of NIBP conditional knockout mice"
The goal of this proposal is to generate NIBP conditional knockout mice.

BC101520 Wenhui Hu, Ph.D. (PI) 4/1/2011-3/31/2013
DOD/BCRP

“Novel function of NIBP in Breast Cancer”
The goal of this proposal is to identify the novel function and mechanisms of NIBP in the tumorigenesis and metastasis of breast cancer cells.

R21 NS062369-A1 (P.I.: Dong Sun, Ph.D.) 06/01/08 - 05/30/10
NIH/NINDS
"Behavior and plasticity of grafted adult human neural stem cells in injured CNS"
The goal of this project is to evaluate the therapeutic potential of adult human neural stem cells isolated from neurosurgical resection tissues for injured brain.

Role: Co-Investigator

R01 DK15564, Karnam S. Murthy, Ph.D. (PI) 04/01/06-3/31/11
NIH/NIDDKD
“Hormonal Integration of Exocrine and Endocrine Organs”
The objective of this project is to characterize the intracellular signaling pathways that mediate the initial and sustained contraction and MLC_{20} *phosphorylation in gastric and intestinal smooth muscle.*
Role: Co-Investigator

RO1 DK28300, Karnam S. Murthy (PI) 04/01/04-3/31/09
NIH, NIDDKD
“Function of Gut Peptides in Isolated Gut Muscle Cells”
The objective of this project is to characterize the signaling pathways that mediate agonist (VIP/PACAP) and nitric oxide (NO)-dependent relaxation in gastric and intestinal smooth muscle.
Role: Co-Investigator