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:** The Trustees of the University of Pennsylvania

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):** Gearline R. Robinson-Hall, BSF

4. **Grant Contact Person's Telephone Number:** 215-746-6821

5. **Grant SAP Number:** 4100050912

6. **Project Number and Title of Research Project:** 12 - Development of High Throughput Methods for Generation of Induced Pluripotent Stem Cells

7. **Start and End Date of Research Project:** 7/1/2011 - 6/30/2013

8. **Name of Principal Investigator for the Research Project:** John D. Gearhart, 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:

   $ $930,475.81

   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
Addis, Russell	Sr. Research Investigator		$ 916.66
Arbelaez, Juan	Research Specialist	50%	$19,275.34
Liu, Ying	Research Assistant	21%	$ 5,661.92
Lu, Jenny	Lab Technician	100%	$ 568.00
Morrisey, Ed	Project Director	9%	$17,589.50
Oliva, Andrea	Research Assistant	100%	$ 3,685.00
Stewart, Kathleen	Sr. Research Investigator	54%	$47,986.00
Stout, Matthew	Research Specialist	41%	$28,365.66
Yang, Wenli	iPS Director, Co-Investigator	18%	$63,795.07
Yan, Ruilan	Research Specialist	10%	$ 7,275.60

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
Gearhart, John	Principal Investigator	<1%

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
Heracell 150I Copper CO_2 Incubator	Growing cells	$13,218.02
A2 Class II Biosafety Cabinet	Passaging hESC/iPSC cultures	$28,451.76
Nikon TSF-100 TC Microscope w/ Attachments	Observing cells	$ 5,818.43
Revco Freezer 400BX 115V ULT w/ Racks	Storage of lab reagents	$ 9,806.40
Leica M125 Microscope and Leica TL4000 Lightbase	Picking iPSC colonies	$28,613.12
Eppendorf 5810R Centrifuge	Centrifugation for experiments	$ 7,664.19
Dual Heracell CO_2/O_2 CU 120V Incubators	Growing cells	$16,449.56
Thermo 600BX 115V ULT w/ Racks	Storage of lab reagents	$12,239.74
Eppendorf Centrifuges 5702R	Centrifugation for experiments	$ 5,110.71
Thermo Hera150I Trigas STK Cu 120	Growing cells	$36,643.40

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:
U01-HG006398
NIH/NHLBI
Co-PI's: Daniel J. Rader, Edward E. Morrisey, and Stephen A. Duncan
iPS-derived hepatocytes to study lipid phenotypes associated with GWAS lipid loci
7/1/11 – 6/30/16 $100,000

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_____ No_____X_____

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

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

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

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

Yes____X____ No_____

If yes, please describe your plans:

We plan to apply for additional NIH or private funding pending submission/publication of our project.

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

We will finish characterizing the iPS cell pools generated using our batch method in Aim 2 of this project as described in section 17 of this report. Our plan is to publish our work describing the generation and characterization of iPS cells by the batch purification method.

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

Yes_____ No____X____

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

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

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

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

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

Yes____X____ No_____

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

Juan Arbelaez, BS Biochemistry and Cell Biology
University of California, San Franciso

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.

We were able to use the Pennsylvania Department of Health - Health Research Formula Funds (HRFF) to purchase capital equipment and to hire new personnel in order to drastically expand the induced Pluripotent Stem Cell (iPSC) Core facility in our new location. As a result, we have been able to expand iPSC services to investigators at University of Pennsylvania and provide an invaluable resource for human embryonic stem cell and iPS cell related research. In the two years we were funded by the HRFF, the iPSC Core has worked with more than a dozen investigators on campus, providing services and expertise. Several of the projects that we supported have led to publications and/or grant funding.

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:

Dr. Stephen Duncan
Medical College of Wisconsin

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:

The epigenetic reprogramming of somatic cells to induced pluripotent stem cells (iPSCs) by expression of a set of transcription factors is a major advance in stem cell research. iPSC technology has become as a key research tool for disease modeling. Although significant

advancement has been made in the iPSC field, current methods for generating iPSCs are labor intensive and inefficient and therefore do not afford large-scale studies where iPS cells need to be derived from hundreds of patient samples. In addition, development of efficient transgene-free approaches is needed in order to derive iPSCs that are amenable to downstream studies as well as clinical applications. To address these issues, we propose the following research aims in this project to optimize and develop new methods for iPSC derivation:

1. We will optimize the generation of functional iPSCs from human adipose stem cells (ASCs) using current methods as well as a novel approach that utilizes miRNA-mediated iPSC reprogramming. We will generate iPSCs from ASCs using a polycistronic lentiviral vector expressing the 4 transcription factors as well as a lentiviral vector expression miR302/367 to optimize feeder-dependent and feeder-free iPSC derivation protocols.

2. We will develop methods to successfully overcome the hurdles in generating large numbers of iPS cells. To this end, we propose to generate and characterize pools of iPSCs and compare this technique to using individual clones in order to bypass the need to pick individual clones. We will also attempt to increase throughput by miniaturizing iPSC derivation using 24-well plates and selecting iPSCs with antibodies against cell surface pluripotency markers.

3. We will develop transgene-free iPSC derivation methods using miRNA mimics against miR302/367, which we have shown to efficiently reprogram iPS cells.

During the 2-year funding period of this project, we have achieved most of the goals that we have outlined or have redirected our goals due to results obtained while addressing those goals.

<u>Aim 1: Optimization of technology for generation of iPSCs from human adipose stem cells (ASCs)</u>

We proposed to optimize a protocol for efficient derivation of iPSCs from ASCs. This is driven by published reports showing increased efficiency of reprogramming using ASCs as well as a large ongoing project with Dan Rader's group at University of Pennsylvania (UPenn) involving the use of ASCs for iPSC reprogramming. We have optimized a working protocol that uses 1-2x10^5 early passage ASCs (p2-p3) derived from subcutaneous adipose biopsies and the polycistronic lentiviral vector hSTEMCCA-hloxp at a MOI of 10 for reprogramming. We are able to achieve an efficiency of 0.01-0.5% and a reprogramming rate of about 3-4 weeks, both of which parameters are comparable to other published reports using ASCs. We also observed lentiviral transgene silencing in the iPS cells after serial passaging, which suggests that transgene excision may not be necessary for subsequent differentiation (data not shown). Using this method, we have generated and characterized 20-24 iPS lines from 8 individuals. These iPSC lines express high levels of pluripotency markers and are able to form embryoid bodies comprising cells of all three lineages (Fig.1). Some of these lines have been successfully

differentiated into hepatocytes (endoderm) and endothelial cells (mesoderm, data not shown) in collaboration with Steve Duncan and Muredach Reilly's groups.

For a better flow of the report, we will discuss Aim 3 before Aim 2.

Aim 3 -- Development of iPSC generation free of recombinant methods:

We proposed the generation of footprint-free iPSCs using micro RNA mimics. However, the inherent instability and the low reprogramming efficiency of the mimics quickly redirected us to other methods. We optimized a protocol using Sendai viral vectors (SeV) and peripheral blood mononuclear cells (PBMCs) for efficient footprint-free generation of iPSCs. Sendai viruses are cytoplasmic replicating RNA viruses that do not integrate into the host genome, thus ideal for footprint-free iPSC generation. In addition, recent advances in using peripheral blood for iPSC reprogramming highlights the advantage of this non-invasive and easy tissue-sampling alternative to skin or adipose biopsies. We therefore decided to switch our starting somatic cell source from ASCs to PBMCs. We first isolate PBMCs from a small amount of blood (4-8 mls); the PBMCs are cultured next for expansion of erythroid progenitors for 7-10 days in the presence of a defined cytokine cocktail, which are then used for iPSC reprogramming with Sendai viral vectors (Fig. 2). We are able to consistently achieve a high reprogramming efficiency of 0.01% -0.5% and an increased rate of reprogramming of 2-3 weeks. The iPSC lines express high levels of all pluripotency markers tested (Fig. 2). We have successfully generated iPS cell lines from over 50 blood samples, with >95% success. This protocol serves as our Standard Operating Procedure for iPSC generation in the UPenn iPSC Core facility. It also has been adopted by several other stem cell core facilities (e.g. Mount Sinai School of Medicine, Harvard Stem Cell Institute) for iPSC generation. It has been published in an online open access format at Stembook.org (http://www.stembook.org/node/765).

One hallmark of high quality iPSCs is their ability for efficient differentiation into the cell lineage of interest. We have subjected iPSCs derived using this method for generating hepatocytes (endoderm), vascular endothelial cells (mesoderm) and macrophages (mesoderm) in collaboration with Steve Duncan and Muredach Reilly's groups. These iPSCs (SV7, SV10, SV20) are able to efficiently differentiate into hepatocytes that express key markers of mature cells such as albumin and HNF4α (Fig. 3) at high levels, comparable to a previously published control iPSC line K3. These iPS cells are also able to differentiate into macrophages and vascular endothelial cells robustly (data not shown). We have thus shown that iPSCs derived using our method are able to generate cell types of endoderm and mesoderm lineages robustly.

Aim 2 -- Development of methods for high throughput generation of iPSCs

Currently, the iPSC field faces two inherent rate-limiting hurdles that must be overcome in order to allow scale-up generation of large numbers of iPSC lines for population studies: 1) the requirement for manually picking and expanding clones and subsequent characterization; and 2) the lack of miniaturization of the cell culture methods. In order

to develop higher throughput methods, we proposed to use batch or pool generation of iPSCs using rigorous cell surface pluripotent marker(s) selection followed by additional characterization for pluripotency. To this end, we reprogrammed a blood sample using both Sendai virus and the integrating STEMCCA virus so that we can follow the transgene in the iPS cells. We picked individual clones and expanded them per standard protocol. Concurrently, we also batch selected for pluripotent stem cells from a duplicate plate by labeling cells with TRA-1-60 or SSEA-4 antibodies followed by magnetic activated cell sorting (MACS). To enrich for pluripotent cell populations, we performed 2 to 3 rounds of antibody selection over 3-4 passages. We found that by applying this method, we were able to generate homogenous iPS cell pools with similar surface antigen expression profile as compared to iPSC clones, and the iPS cell pools maintain stable and high level expression of pluripotency markers through passages as high as 60s and 70s (Fig. 4). The SVpool cells also exhibit a normal karyotype with serial passaging (data not shown).

An important question to address is whether pooled iPS cells are able to differentiate as well as clones. To this end, we collaborated with Steve Duncan's group to perform directed differentiation into hepatocytes. We showed that pooled cells converted into high albumin and HNF4α producing cells as efficiently as clones, both at lower (p30s) and high passages (p80s, Fig. 3). We also compared pools and clones for the ability to differentiate into all three lineages *in vitro* by embryoid body (EB) formation assay, followed by gene expression analysis of 74 lineage genes by qRT-PCR. Data analysis showed no significant differences in the expression levels of the lineage genes in the clones and pool, suggesting that the SVpool cells are able to differentiate into all three lineages as well as the clones (data not shown).

Another important question we sought to address is whether the iPSC pool stays multi-clonal over serial passaging. To this end, we analyzed the iPSC pool (STpool) and several clones that we generated using the STEMCCA lentiviral reprogramming vector. STEMCCA integrates into the iPSC genome, usually one copy per cell, thus allowing us to follow each individual clone in the STpool by Southern Blotting. We performed Southern Blotting on genomic DNA isolated from cell cultures from early to late passages (p4-p33). We saw multiple bands in the STpool lanes, indicating multiple clones, and these bands did not diminish appreciably over passaging or collapse into one band (Fig. 5). These results suggest that iPSC pools generated by the above method appear to stay multi-clonal, at least through the passages examined. To better quantify this result, we are in the process of cloning the STEMCCA insertion sites in the iPS cell pools by PCR, followed by high throughput DNA sequencing. This will allow us to determine the numbers of unique clones remaining over long term serial passaging in the cell pools. We are in the process of preparing a manuscript describing the generation and characterization of iPS cells by the batch purification method.

In addition to supporting the research activities described above, a portion of this funding also provided salary and reagent support for the operation of the iPSC Core facility for generation and characterization of iPSCs for researchers at UPenn.

Publications

Yang, W., Mills, J. A., Sullivan, S., Liu, Y., French, D. L., and Gadue, P (2012) iPSC reprogramming from human peripheral blood using Sendai Virus mediated gene transfer. Stembook [internet]. Cambrige (MA): Harvard Stem Cell Institute; 2012 December 11

Note from Dr. Wenli Yang: This was not a publication in a peer-reviewed journal and had no official format to include citation of funding sources, therefore it was not included.

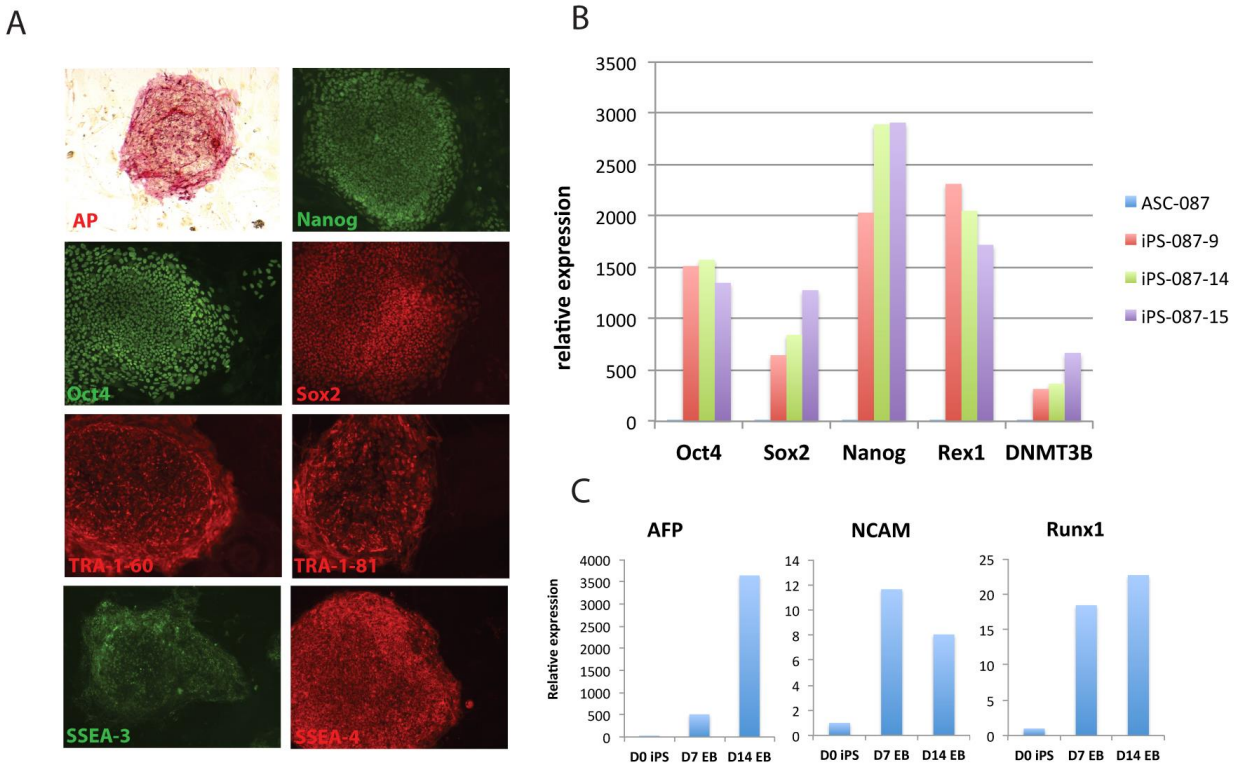

Fig. 1. iPSCs generated from ASCs using STEMCCA lentiviral vector are pluripotent and form embryoid bodies. A). Immunostaining of iPS-087-15 cells showing expression of a panel of pluripotency markers. B). qRT-PCR analysis showing three iPSC lines express high levels of pluripotency markers compared to the corresponding ASCs. C). qRT-PCR analysis showing day 7 and day 14 embryoid bodies generated from iPS-087-15 cells express markers of all three germ layers (AFP: endoderm, NCAM: ectoderm, Runx1: mesoderm)

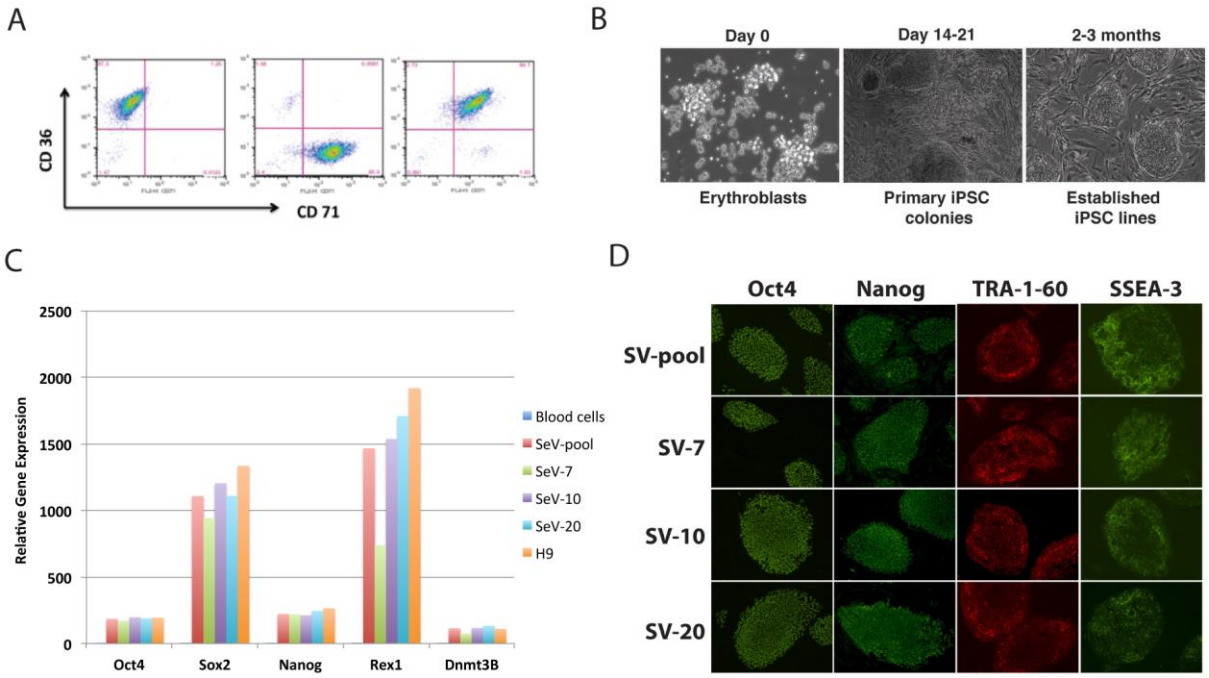

Fig. 2. Generation of footprint free iPSCs from blood using Sendai viral vectors. A). FACS analysis of a PBMC sample that has been expanded for enrichment of erythroblasts. >90% of cells are positive for erythroid progenitor markers CD36 and CD71 after 9 days of expansion. B). Phase contrast pictures of iPS generation from erythroblasts at different time points after viral infection. C). qRT-PCR analysis of RNA from established iPS clones and pools. Both pooled cells and clones show high levels of pluripotency marker expression compared to the control hESC line H9. D). Immunostaining of pluripotency markers of iPSCs in C showing expression of pluripotency markers.

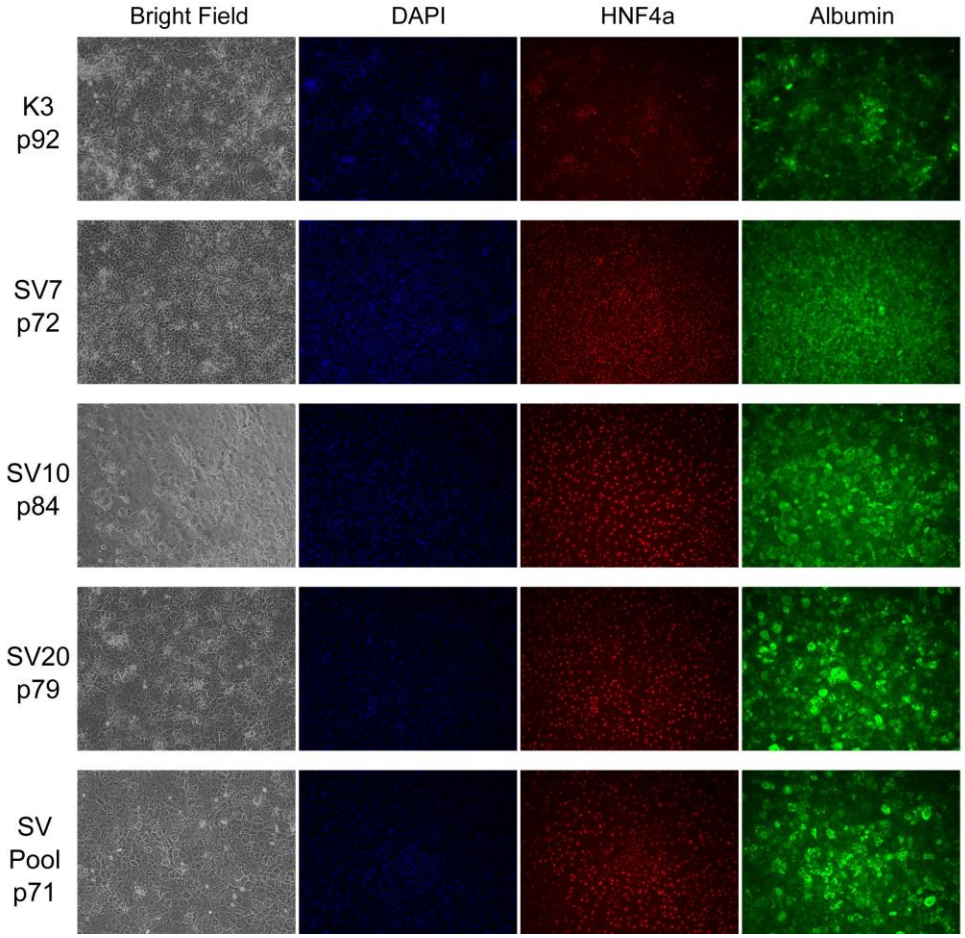

Fig. 3. Immunofluorescence pictures of iPSC derived hepatocytes showing high expression of hepatic markers in Day 20 differentiated cells. K3: control iPSC line for efficient hepatocyte differentiation; SV7, SV10, SV20, SVPool: Blood derived iPSC clones and cell pool using Sendai virus.

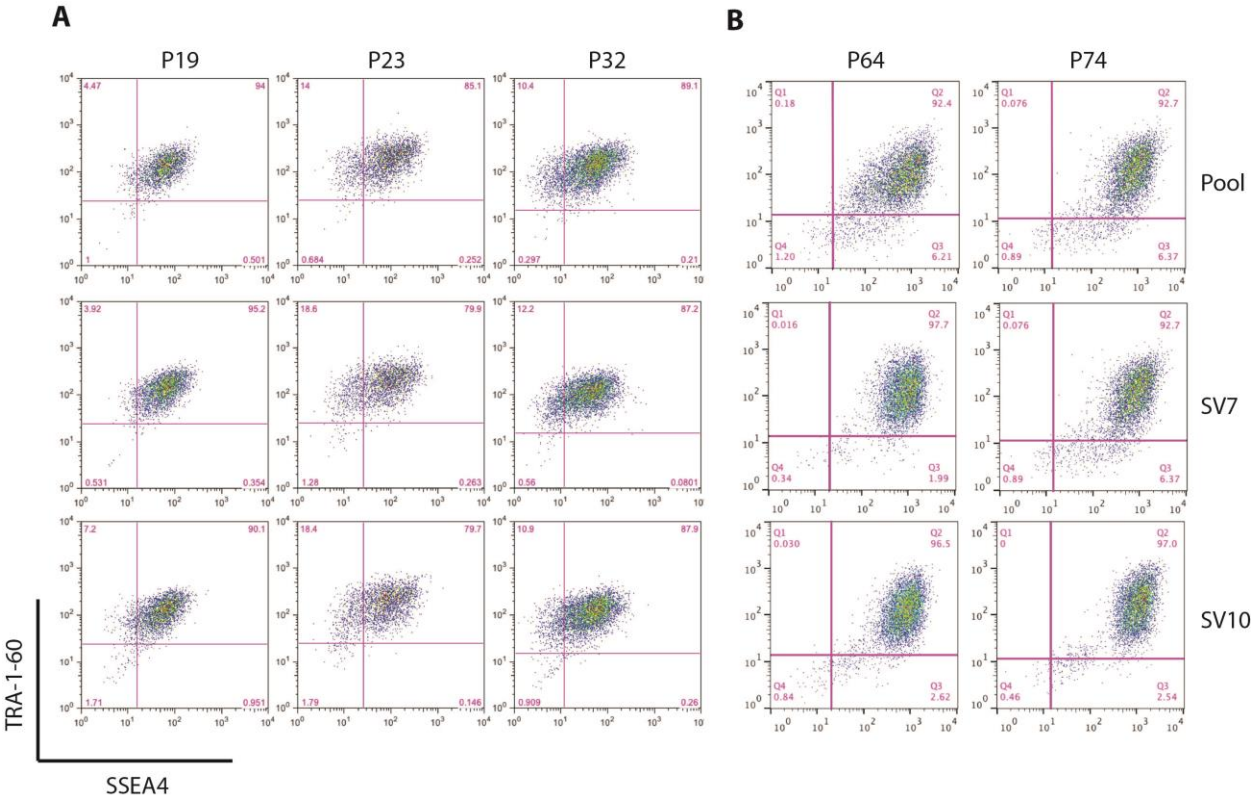

Fig. 4. FACS analysis of established iPSC clones (SV7, SV10) and cell pool (Pool). Both pool and clones maintain stable and high levels of SSEA4 and TRA-1-60 expression through serial passaging.

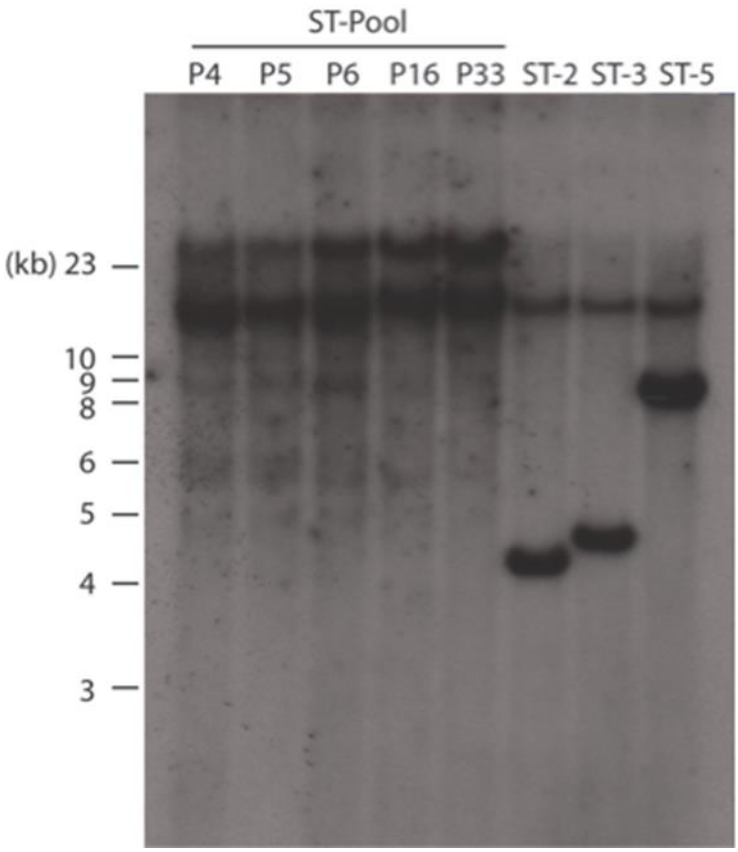

Fig. 5. Southern Blotting analysis of genomic DNA prepared from iPS cell pool (ST-Pool) and clones (ST-2, ST3, ST-5) using a DNA sequence (WPRE) from the lentiviral reprogramming vector backbone as the probe. DNA from passage 4, 5, 16 and 33 ST-Pool cells were analyzed as well as DNA from passage 15 of each of the iPSC clones.

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?
___X__Yes
_____ No

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

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

We used the NIH approved H9 human embryonic stem cell as a control in our studies.

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. None				☐Submitted ☐Accepted ☐Published

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

Yes____X____ No_____

If yes, please describe your plans:

We are in the process of preparing a manuscript and will soon submit to a peer-reviewed journal.

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

None.

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

None.

23. Inventions, Patents and Commercial Development Opportunities.

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

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

a. Title of Invention:

b. Name of Inventor(s):

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

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

John D. Gearhart, Ph.D., is the James W. Effron University Professor and is the Director of the Institute for Regenerative Medicine at the University of Pennsylvania. He is a developmental geneticist and his research over the past several decades has been directed at an understanding the molecular and cellular basis of human embryonic development. Dr. Gearhart is a leader in the development and use of human reproductive technologies, embryo and germ cell manipulations and in the genetic engineering of cells. In 1998, Dr. Gearhart and his research team at Johns Hopkins published the first report on the derivation of pluripotent stem cells from germ cells of the human embryo. These cells have the capacity to form all cell types and tissues present in the human body and are considered a major starting point for the development of a wide variety of cell-based therapies in the new field of regenerative medicine. His research is focused on the basic science of stem cells, stem cell specialization, and the generation of cell-based therapies for a number of diseases and injuries.

Dr. Gearhart was a founding member of the International Society for Stem Cell Research and serves on a number of advisory boards and committees of foundations, institutes and professional societies involved in stem cell research and policy and science outreach and has served as a consultant or expert witness for many governmental agencies, in states, at the national level and to governments of foreign countries. He currently serves on the FDA Advisory Committee for Cellular, Tissue and Gene Therapies and the World Anti-Doping Authority, Gene Doping Expert Group.

BIOGRAPHICAL SKETCH

NAME Wenli Yang, PhD	POSITION TITLE Director, induced Pluripotent Stem Cell (iPSC) Core Facility, University of Pennsylvania
eRA COMMONS USER NAME (credential, e.g., agency login)	

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
University of Illinois-Urbana, IL	B.S.	1991-1995	Biochemistry
University of California-Los Angeles, CA	Ph.D.	1995-2001	Signal transduction/Ras Signaling
Harvard Medical School, Boston, MA	Post-doc	2001-2008	Transcriptional control of adipogenesis

A. Personal Statement

I direct the induced Pluripotent Stem Cell (iPSC) Core Facility within the Institute for Regenerative Medicine at the University of Pennsylvania. Our mission is to promote iPSC/stem cell technology at UPenn. To this end, we have established standard protocols for derivation of iPSCs from somatic cells. We work with investigators to derive and characterize disease-specific iPSCs as cellular models of disease. We also perform genome editing in human embryonic stem cells (hESCs) and iPSCs using CRISPR and TALEN technologies. We provide expertise and training to researchers in hES/iPS cell culture. In addition, we serve as a resource for sharing iPS cell lines and iPSC technology within the UPenn and the broader scientific community. We continue to optimize new protocols and bring new technologies to the Penn campus.

B. Positions and Honors

Positions

1995-2001 Ph.D. Candidate, University of California, Los Angeles
2001-2008 Postdoctoral Fellow, Dana Farber Caner Institute and Harvard Medical School, Boston, MA
2008-2009 Scientist, Syndexa Pharmaceuticals Corp., Cambridge, MA
2009-present Director, iPSC Core Facility, Institute for Regenerative Medicine, University of Pennsylvania School of Medicine, Philadelphia, PA

Honors

1995 Hauser Scholarship for Undergraduate Research, University of Illinois
1997 Edwin D. Pauley Foundation Graduate Fellowship, UCLA
2000 PhD. Dissertation Year Fellowship, UCLA
2003 Individual National Research Service Award (NRSA), NIH
2006 Friends of Dana Farber Postdoctoral Research Fellowship, Harvard Medical School

C. Selected peer-reviewed publications (in chronological order).

1. Yang, W., Del Villar, K., Urano, J., Mitsuzawa, H. and Tamanoi, F. (1997) Advances in the development of farnesyltransferase inhibitors: substrate recognition by protein farnesyltransferase. *J. Cell. Biochem.* S27, 12-19.
2. Yang, W., Urano, J., and Tamanoi, F. (2000) Protein farnesylation is critical for maintaining normal cell morphology and canavanine resistance in *Schizosaccharomyces pombe*. *J. Biol. Chem.* 275, 429-438.
3. Tamanoi, F., Del Villar, K., Robinson, N., Kim, M.R., Urano, J., and Yang, W. (2000) Genetic analysis of FTase and GGTase I and natural product farnesyltransferase inhibitors, in *Farnesyltransferase Inhibitors in Cancer Therapy* (Sebti, S. M., Hamilton, A., eds.). Humana Press Inc., Totowa, NJ.
4. Urano, J., Yang, W., and Tamanoi, F. (2000) Mutational analyses of protein farnesyltransferase, in *Protein Lipidation* (Tamanoi, F., Sigman, D., eds.). The Enzymes, Third Edition, Academic Press, San Diego.
5. Urano, J., Tabancay, A. P., Yang, W., and Tamanoi, F. (2000) The *Saccharomyces cerevisiae* Rheb G-protein is involved in regulating canavanine resistance and arginine uptake. *J. Biol. Chem.* 275, 11198-11206.
6. Yang, W., Tabancay, A. P., Urano, J., and Tamanoi, F. (2001) Failure to farnesylate Rheb protein contributes to the enrichment of G0/G1 phase cells in the *Schizosaccharomyces pombe* farnesyltransferase mutant. *Mol. Microbiol.* 41, 1339-1347.
7. Mootha V. K., Handschin, C., Arlow, D., Xie, X., St. Pierre, J., Sihag, S., Yang, W., Altshuler, D., Puigserver, P., Patterson, N., Willy, P. J., Schulman, I. G., Heyman, R. A., Lander, E. S., and Spiegelman, B. M. (2004) Erralpha and Gabpa/b specify PGC-1alpha-dependent oxidative phosphorylation gene expression that is altered in diabetic muscle. *PNAS* 101, 6570-6575.
8. Lin, J., Yang, R., Tarr, P. T., Wu, P.-H., Handschin, C., Li, S., Yang, W., Pei, L., Uldry, M., Tontonoz, P., Newgard, C. B., and Spiegelman, B. M. (2005) Hyperlipidemic effects of dietary saturated fats mediated through PGC-1beta coactivation of SREBP. *Cell* 120, 261-273.
9. Rhee, J., Ge, H., Yang, W., Fan, M., Handchin, C., Cooper, M., Lin, J., Le, C., and Spiegelman, B. M. (2006) Partnership of PGC-1 and HNF4 in the regulation of lipoprotein metabolism. *J. Biol. Chem.* 281, 14683-14690.
10. St-Pierre, J., Drori, S., Uldry, M., Rhee, J., Jager, S., Lin, J., Handschin, C., Yang, W., Bachoo, R., and Spiegelman, B. M. (2006) Suppression of reactive oxygen species and MPTP-induced neurodegeneration by the PGC-1 transcriptional coactivators. *Cell* 127, 397-408.
11. Cooper, M. P., Qu, L., Rohas, L. M., Lin, J., Yang, W., Erdjument-Bromage, H., Tempst, P., and Spiegelman, B. M. (2006) Defects in energy homeostasis in Leigh syndrome French Canadian variant through PGC-1alpha/LRP130 complex. *Gene & Dev.* 20 (21), 2996-3009.
12. Sandri, M., Lin, J., Handschin, C., Yang, W., Arany, Z. P., Lecker, S. H., Goldberg, A. L., and Spiegelman, B. M. (2006) PGC-1alpha protects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription. *PNAS* 103 (44), 16260-16265.
13. Uldry, M., Yang, W., St-Pierre, J., Lin, J., Seale, P., and Spiegelman, B. M. (2006) PGC-1 coactivators control fat thermogenesis, mitochondrial biogenesis but not cell differentiation. *Cell Metab.* 3 (5), 333-341.
14. Arany, Z., Lebrasseur, N., Morris, C., Smith, E., Yang, W., Ma, Y., Chin, S., and Spiegelman, B. M. (2007) The transcriptional coactivator PGC-1beta drives the formation of oxidative type IIX fibers in skeletal muscle. *Cell Metab.* 5(1), 35-46.
15. Seale, P., Kajimura, S., Yang, W., Chin, S., Rohas, L. M., Uldry, M., Tavernier, G., Langin, D., and Spiegelman, B. M. (2007) Transcriptional control of brown fat determination by PRDM16. *Cell Metab.* 6(1), 38-54.
16. Seale, P., Bjork, B., Yang, W., Kajimura, S., Kuang, S., Scime, A., Devarakonda, S., Chin, S., Conroe, H.M., Erdjument-Bromage, H., Tempst, P., Rudnicki, M.A., Beier, D.R., and

Spiegelman, B.M. (2008). PRDM16 Controls a Brown Fat/Skeletal Muscle Switch. Nature, 454, 961-967.

17. Anokye-Danso, F., Trivedi, C. M., Juhr, D., Gupta, M., Cui, Z., Tian, Y., Zhang, Y., Yang, W., Gruber, P.J., Epstein, J. A., and Morrisey, E. E. (2010) Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell, 8(4),376-88.
18. Yang, W., Mills, J. A., Sullivan, S., Liu, Y., French, D. L., and Gadue, P (2012) iPSC reprogramming from human peripheral blood using Sendai Virus mediated gene transfer. Stembook [internet]. Cambrige (MA): Harvard Stem Cell Institute; 2012 December 11.