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 Institute for Cancer Research

2. **Reporting Period (start and end date of grant award period):** 1/1/2012 – 6/30/2014

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Maria Minko Gill

4. **Grant Contact Person's Telephone Number:** 215-728-2659

5. **Grant SAP Number:** 4100057660

6. **Project Number and Title of Research Project:** 01- Impacts of Inhibitory Receptors on Signaling Competence of NK Cells

7. **Start and End Date of Research Project:** 1/1/2012 – 12/31/2013

8. **Name of Principal Investigator for the Research Project:** Kerry S. Campbell, 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:

   $431,764

   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
Campbell, Kerry	PI	20% years 1-2	$53,046.81
Oshinsky, Jennifer	Scientific Technician II	35% years 1-2	$34,920.95
MacFarlane, Alexander	Research Associate	32% years 1-2	$46,698.34
Purdy, Amanda	Research Associate	25% years 1-2	$36,445.17

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
None		

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

Type of Scientific Equipment	Value Derived	Cost
None		

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

Yes_____ No____X_____

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

11. Leveraging of Additional Funds

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

Yes_____X____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If

you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

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

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds awarded:
R01 - Molecular Mechanisms Regulating the Signaling Competence of NK Cells	☒ NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	February 2013	$2,231,250	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:

Will resubmit a revised grant application to the NIH in the near future.

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

We are developing relationships with a new bone marrow transplantation team at Fox Chase Cancer Center/Temple University to obtain and study signaling competence of human NK cells from donors and transplant recipients. These efforts will improve the clinical relevance of the project and thereby increase significance of the studies.

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

Yes_____ No_____X_____

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

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

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

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

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

Yes_____ No_____X_____

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

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

Yes_____ No_____X_____

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

Yes, we contributed to a collaborative project with Dr. Jordan Orange, formerly of University of Pennsylvania (now at Baylor University in Houston). This work led to a publication in the *Journal of Clinical Investigation*.

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.

Natural killer (NK) cells can distinguish normal cells from tumor cells through signals derived from activating and inhibitory receptors. In mature NK cells, inhibitory receptors [Ly49 in mice and killer cell Ig-like receptors (KIR) in humans] are key regulators that prevent attack of normal cells through their detection of major histocompatibility complex class I (MHC-I) molecules on virtually every normal cell of the body. Tumor cells are often MHC-I-deficient and attract strong NK cell attack by lacking the inhibitory receptor ligands. Ly49, KIR, and their MHC-I ligands are encoded by highly polymorphic families of genes, and some inhibitory receptors are expressed in an individual that do not recognize the endogenous MHC-I, which is considered "self". Different inhibitory receptors are stochastically expressed during NK cell development, and the expression of a self-recognizing inhibitory receptor (SRIR) paradoxically promotes the maturation of a functionally competent NK cell that can elicit cytotoxicity and cytokine production. In contrast, NK cells lacking SRIR are non-functional, and it has been proposed that these cells are anergic due to chronic activation signaling in the absence of inhibitory signaling. Supporting this hypothesis, we have previously reported that NK cells in mice lacking the activation signaling adaptor protein, B cell adaptor for phosphatidylinositol 3-kinase (BCAP), are more functional and more mature, even in a MHC-I deficient environment. A major gap in understanding, however, is how expression of SRIR impacts the competence of signal transduction through NK cell activating receptors and whether maturation plays a role in signaling competence. Our primary goal of this project was to improve understanding of the intertwining roles of SRIR and maturation in promoting signaling and functional competence of NK cells in mice and humans. We addressed the following specific aims:

1. How do inhibitory Ly49 impact the development of signaling competence in mouse NK cells?
2. How do inhibitory KIR impact the development of signaling competence in human NK cells?

We had previously established a non-invasive method to assess calcium signaling of resting NK cell subpopulations from freshly isolated splenocytes by flow cytometry. To improve this assay system by further minimizing cell manipulation, we employed splenocytes from heterozygous $Ncr1^{gfp/+}$ mice, in which all NK cells are tagged with EGFP. A single cell suspension of fresh $Ncr1^{gfp/+}$ splenocytes was loaded with indo-1, then labeled with fluorophore-conjugated mAbs to Ly49 and NKG2A IR, and stained with propidium iodide. Cells were then mixed with biotinylated anti-NK1.1 (NKR-P1C) mAb and immediately subjected to flow cytometry. After establishing a baseline of intracellular calcium level in the viable $EGFP^+$ NK cells, streptavidin was added to crosslink NK1.1 and induce robust calcium mobilization in the NK cells. We further delineated responses in NK cell subpopulations by gating on expression or lack of various

self-recognizing inhibitory receptors (SRIR), which engage with endogenous MHC class I molecules, and unligated IR, which lack available ligand. Importantly, surface expression of NK1.1 did not differ on the NK cell subsets analyzed. The mean relative intracellular calcium concentrations above baseline were then compared for these unique NK cell subsets from different mice assayed under identical conditions.

Using this assay system for Subaim 1a, we gated on NK cells expressing or lacking the SRIR that educate NK cells in C57BL/6 mice (Ly49C, Ly49I, and NKG2A) and an unligated IR, Ly49G2. Cells were first gated on expression or lack of Ly49G2, which is expressed on about half of the NK cells. Within the Ly49G2^{-} population, NK cells lacking any SRIR expression were least responsive, but populations expressing SRIR demonstrated significantly greater calcium responses, with the strongest response in NK cells co-expressing both NKG2A and Ly49C/Ly49I (Fig. 1). Our results demonstrate that SRIR expression promotes calcium signaling competence, consistent with its previously reported impact on function (licensing/arming IFN-γ and cytotoxicity responses), whereas lack of SRIR results in reduced calcium signaling competence, consistent with "disarming" at the level of or upstream of calcium signaling. In contrast, NK cells from H-2K$^{b-/-}$D$^{b-/-}$ mice were incompetent to mediate calcium signaling in response to NK1.1 crosslinking (not shown; addresses Subaim 1d), which further supports the disarming hypothesis, whereby cells lacking SRIR are hyporesponsive due to chronic activation signaling.

When gating on SRIR^{+} NK cells, the strongest calcium responses were observed in cells lacking expression of any unligated IR. Co-expression of one unligated IR significantly reduced calcium signaling, and expression of more than one unligated IR diminished calcium signaling even further, even in MHC-1-deficient mice (Fig. 2). Importantly, these results show that unligated IR expression suppresses calcium signaling in a quantitative manner to dampen overall activation signaling competence. Our results revealed a negative rheostat shaped by the repertoire of unligated IR that dampens NK cell signaling competence and support a model in which non-SRIR may contribute significantly to the hyporesponsive state of SRIR-deficient NK cells.

We also tested the influence of maturation on calcium signaling competence in NK cells expressing or lacking SRIR. C57BL/6 Ncr1$^{gfp/+}$ splenocytes were gated on their expression of SRIR and the maturation markers CD11b and CD27. When gating on the SRIR-expressing NK cells (not shown), immature cells (R1: CD27^{+}CD11b^{-}) were least responsive, but calcium signaling was further enhanced through stage of maturation (R2: CD27^{+}CD11b^{+} and R3: CD27^{-} CD11b^{+}). SRIR-deficient NK cells were significantly less responsive, but also showed increased signaling in the transition from R1 to R3. The weakly responsive NK cells from H-2 K^{b}D$^{b-/-}$ mice also demonstrated elevated calcium signaling through maturation stage. Therefore, we conclude that calcium signaling competence also increases with stage of NK cell maturation.

In further studies to address Subaim 1c, we tested the functional impact of stimulating the different NK cell subsets by testing for interferon (IFN)-γ production by intracellular staining and flow cytometry. In these studies, we found that the primary source of IFN-γ is from NK cells that express SRIR, but are also at a mid-level maturity stage designated by expression of CD11b and CD27 (R2 stage). We conclude from these data that, indeed, part of the "licensing" effect is due to the inherent advanced maturity state of the NK cells that express SRIR. In

contrast, the less functional NK cells lacking SRIR were found to be enriched in less mature NK cells (CD11b-negative $CD27^+$, designated R1). We were, however, surprised that the most mature NK cells ($CD11b^+$ $CD27^-$, R3 stage) did not exhibit the strongest functional responses in these experiments.

In the course of these studies, we also bred and tested BCAP-deficient mice in the calcium studies (Subaim 1d), since we hypothesized that BCAP plays a role in transducing an anergizing activation signal that drives SRIR-deficient NK cells into a hyporesponsive state. BCAP-deficient NK cells responded the same as wild type NK cells, however, in our calcium studies, leading us to conclude that BCAP functions downstream of calcium signaling. We also performed biochemical studies to address the mechanism of BCAP function and found that BCAP associates with several signaling molecules, including Grb2 and TRAF2, which provide strong preliminary data for a grant proposal currently in preparation.

Studies designed to address Subaim 1b were less productive than anticipated. Mouse splenocytes were stimulated as in Subaim 1a for various times, fixed, permeabilized and stained with various phosphoproteins and surface markers to distinguish NK cell subsets. While we were able to show some increased phosphorylation events in anti-NK1.1-stimulated NK cells, the intensity of signal was not adequate to reproducibly compare differential signals in the NK cell subsets, despite significant effort. In contrast, the calcium signaling strength was much higher, resulting in better resolution of differential signaling. Nonetheless, we acquired some preliminary data for future grant proposals through these phospho-specific antibody staining experiments.

For studies addressing Aim 2, we screened a wide array of healthy donors for KIR and HLA-A,B,C ligand expression by flow cytometry and genotyping, in accordance with Subaim 2a. We have used KIR SSP kits from Invitrogen and HLA-B and HLA-C SSP UniTray kits, also from Invitrogen, and we are having these run in our genotyping facility at Fox Chase. In addition, we have begun to establish genomic PCR assays in our laboratory, using oligonucleotide primers that were previously described (Kulkarni et al., Meth. Molec. Biol. 612:365-376, 2010). We focused on KIR2DL1- and KIR3DL1-bearing individuals, since these are both common alleles found in humans, and specific antibodies are available for these KIR (most anti-KIR antibodies are cross reactive with other family members). To date, we have screened about 25 healthy donors in these assays, which provides a wealth of differential expression patterns of KIR2DL1 and/or KIR3DL1, either with or without their ligands. Importantly, most donors expressed both of these receptors, with about one third lacking ligand for each of the receptors.

To address Subaim 2b, we performed calcium mobilization experiments on peripheral blood lymphocytes from some of these donors using techniques established in mouse splenocytes in Subaim 1a. Different stimulating biotinylated antibodies were tested in combination with streptavidin crosslinker to find the best activating signal. Anti-CD16 antibody induced the most robust calcium signal. While anti-NKG2D or anti-NKp46 antibodies induced only barely detectable calcium signals, the combination of anti-NKG2D and anti-NKp46 induced a stronger response that was readily detectable, but less potent than anti-CD16 antibody. Therefore, we focused on the use of anti-CD16 antibody in subsequent studies, which limited our analysis to the more mature $CD56^{dim}$ NK cell subset that predominantly expresses this activating receptor.

When we gated on NK cell subsets, based upon expression of SRIR or unligated IR (focusing on KIR2DL1 and KIR3DL1), we saw some enhanced signaling in NK cell subsets expressing SRIR, but the enhancement was not as pronounced as that found in murine NK cells. Similarly, the reduction of calcium signaling in NK cells expressing unligated IR was less pronounced than in NK cells from mice. While the results were less exciting than expected, the resolution may be hindered by lack of monoclonal antibodies specific for each of the individual inhibitory KIR, specifically KIR2DL2, KIR2DL3, and KIR3DL2. Available antibodies to these inhibitory KIR cross react with activating KIR, which limits their usefulness in fully resolving their impacts in our experiments. Based upon our data, we also hypothesize that calcium signaling in mouse NK cells may be more significantly influenced by SRIR, due to the cis interactions that occur between Ly49 and MHC-I on the surface of mouse NK cells themselves. In contrast, KIR appear to lack capacity to interact with MHC-I in cis on the surface of human NK cells, thereby allowing them to diffuse more freely in the plasma membrane on the surface and more readily and uniformly interact with and blunt early signaling events through NK cell activating receptors.

Meeting Abstracts:
Impacts of Inhibitory Receptor expression and maturation on Signaling Competence of NK Cells. Kerry S. Campbell and Alexander W. MacFarlane IV. Presented at the NK2012: 13th Meeting of the Society of Natural Immunity, Asilomar, CA, April 2012

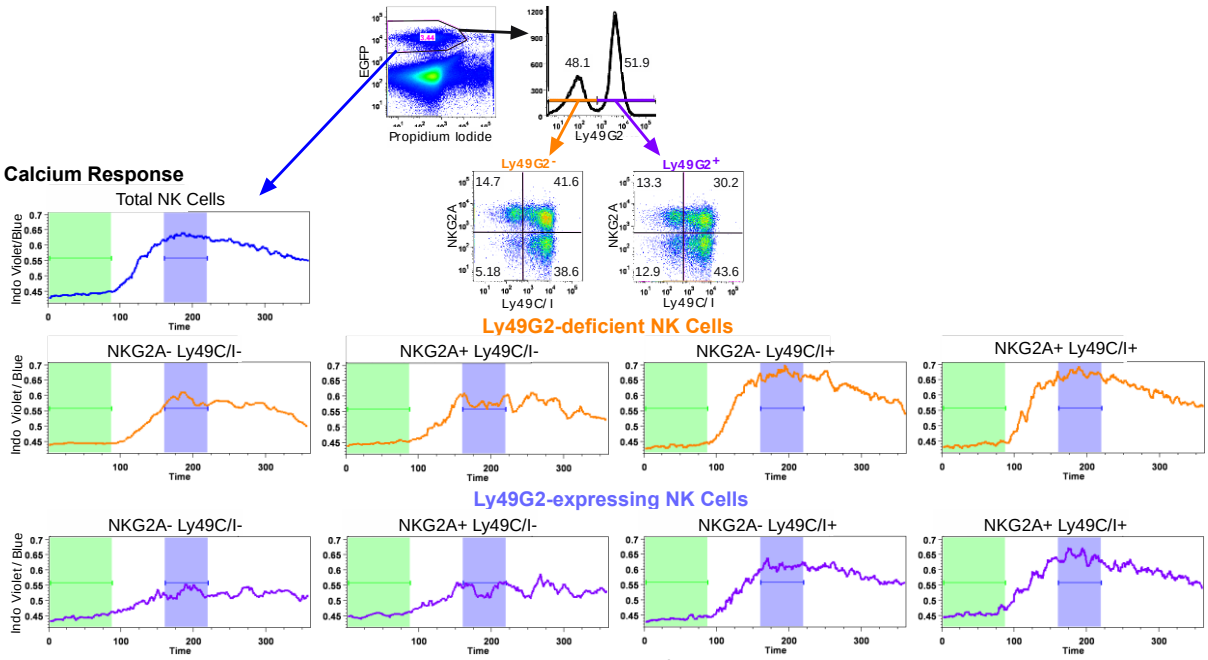

Figure 1. Calcium response of NK cell subsets from Ncr1$^{gfp/+}$ C57BL/6 mice. Splenocytes from C57BL/6 Ncr1$^{gfp/+}$ mice (all NK cells express GFP and NKp46) were stained for SRIR (Ly49C-APC, Ly49I-APC, and NKG2A-PE) or unligated IR (Ly49G2-Cy7-PE), loaded with indo-1, propidium iodide stained, and analyzed for intracellular Ca^{+2} concentration by flow cytometry. Biotinylated anti-NK1.1 mAb was added prior to acquiring baseline Ca^{+2} for 90 sec, streptavidin was added, and response was monitored over time. Viable NK cells were gated as single cells (FSH vs. FSA; not shown) that were propidium iodide-negative and GFP^{+} (top left panel). Ca^{+2} responses were assessed in total NK cells (blue arrow) and NK cell subsets either lacking (orange gate in upper right histogram and Ca^{+2} plots below) or expressing the unligated IR Ly49G2 (purple gate and calcium plots) that were further sub-gated based on SRIR expression (Ly49C/I and/or NKG2A in dot plots below). Relative mean Ca^{+2} over time was based upon indo violet/blue emission of individual cells within each gated NK cell subset. Ca^{+2} was quantified in NK cell subsets during the 90 sec baseline period (green shaded) and a 60 sec interval at the peak Ca^{+2} level in the total NK cell population (blue shaded). The mean baseline value was subtracted from the mean stimulated value within each experiment to calculate the mean value above baseline in subsequent figures.

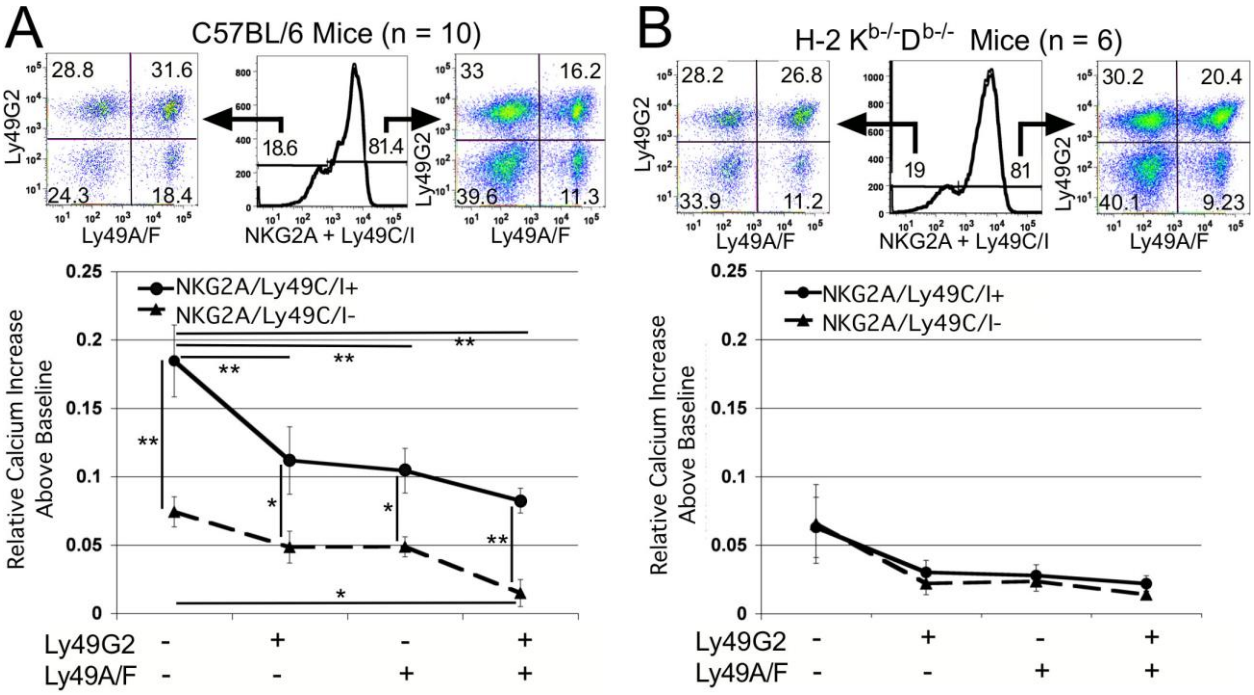

Figure 2. Expression of unligated IR suppresses signaling competence in a dose-dependent manner. Splenocytes from A. Ncr1$^{gfp/+}$ C57BL/6 or B. Ncr1$^{gfp/+}$ K$^{b-/-}$D$^{b-/-}$ mice were stimulated and calcium responses were assessed in NK cell subsets either lacking or expressing SRIR (Ly49C-APC, Ly49I-APC, and NKG2A-APC; middle histogram at top) that were further sub-gated based on expression or lack of unligated IR (Ly49G2-Cy7-PE, Ly49A-PE, and Ly49F-PE in dot plots at top). Mean relative calcium concentration values above baseline were calculated as in Fig. 1. Mean values ±S.E. from indicated number of mice (n) are plotted in bottom panels based on expression (circles) or lack (triangles) of Ly49C/I/NKG2A and expression (+) or lack (-) of Ly49G2 or Ly49A/F (listed below). * designates $p < 0.05$; ** denotes $p < 0.01$ using a paired Student t-test.

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. Human immunodeficiency-causing mutation defines CD16 in spontaneous NK cell cytotoxicity	Jennifer T. Grier, Lisa R. Forbes, Linda Monaco-Shawver, Jennifer Oshinsky, T. Prescott Atkinson, Curtis Moody, Rahul Pandey, Kerry S. Campbell, and Jordan S. Orange	J. Clin. Invest.	May 2012	☐Submitted ☐Accepted ☒Published
2. Natural killer cell biology: An update and future directions	Kerry S. Campbell, Jun Hasegawa	J. Allergy Clin. Immunol.	April 2013	☐Submitted ☐Accepted ☒Published
3. Combination immune therapies to enhance anti-tumor responses by NK cells	Ashley Mentlik James, Adam D. Cohen, and Kerry S. Campbell	Front. Immunol.	Oct 2013	☐Submitted ☐Accepted ☒Published
4. PD-1 Expression on Peripheral Blood Cells Increases with Stage in Renal Cell Carcinoma Patients and Is Rapidly Reduced after Surgical Tumor Resection	Alexander W. MacFarlane IV, Mowafaq Jillab, Elizabeth R. Plimack, Gary R. Hudes, Robert G. Uzzo, Samuel Litwin, Essel Dulaimi, Tahseen Al-Saleem, and Kerry S. Campbell	Cancer Immunol. Res.	Aug 2013	☐Submitted ☐Accepted ☒Published
5. The AP-2 clathrin adaptor mediates endocytosis of an inhibitory killer cell Ig-like receptor (KIR) in human NK cells	Amanda K. Purdy, Diana A. Alvarez-Arias, Jennifer Oshinsky, Ashley M. James, Ilya Serebriiskii, and Kerry S. Campbell	J. Immunol.	Dec 2013	☐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 currently in the final stages of preparing a manuscript summarizing the research that was supported by this CURE funding. This manuscript will be submitted to the Journal of Immunology in the near future.

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 findings were most applicable to understanding the functioning of mouse NK cells, where we identified a differential impact of Ly49 inhibitory receptors that we attribute to their capacity to interact with MHC-I on the NK cell surface in cis, thereby limiting Ly49 from interacting and suppressing the function of activating receptors in the cell membrane. Since the major inhibitory receptors in human NK cells, KIR, do not appear to interact with MHC-I in cis, these influences on calcium signaling are less pronounced. Therefore, the application of our findings to human disease are currently limited. Nonetheless, the findings provide preliminary evidence that serve as a foundation for leveraging additional funds from NIH grant applications already submitted or in preparation.

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.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/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	POSITION TITLE
Campbell, Kerry S., Ph.D.	Associate Professor
eRA COMMONS USER NAME (credential, e.g., agency login) KSCAMPBELL	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
University of Toledo, Toledo, OH	B.S.	06/83	Pharmacy
Medical College of Virginia, Virginia Commonwealth University, Richmond, VA	Ph.D.	02/88	Pharmacology and Toxicology

A. PERSONAL STATEMENT

I have 25 years of research experience in the study of immune cell biology and signal transduction and 15 years of experience in NK cell research. My research has focused primarily on the signaling functions of receptors on these lymphocytes and their impacts on the biological responsiveness. My early work on B cells involved some of the first studies identifying the protein components of the B cell antigen receptor, known as Ig-α and Ig-β. A major current research focus in my lab is the function of killer cell Ig-like receptors (KIR), which are key polymorphic regulators of NK cell activation and tolerance. In addition, we are currently studying NK cell phenotype and function in cancer patients, and NK cell response in a mouse pancreatic cancer model.

B. POSITIONS

Ph.D. Candidate, Department of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA. Advisor: Albert E. Munson, Ph.D.	1983-1988
Postdoctoral Fellow, Department of Pediatrics, National Jewish Center for Immunology and Respiratory Medicine, Denver, CO. Mentor: John Cambier, Ph.D.	1988-1992
Independent Scientific Member, Basel Institute for Immunology, Basel, Switzerland	1992-1998
Associate Member, Fox Chase Cancer Center, Philadelphia, PA	1998-2004
Director, Cell Culture Facility, Fox Chase Cancer Center, Philadelphia, PA	2000-present
Associate Professor, Fox Chase Cancer Center, Philadelphia, PA	2004-present
Adjunct Associate Professor, Department of Microbiology and Immunology, Thomas Jefferson University Medical and Graduate Schools, Philadelphia, PA.	2006-present
Adjunct Associate Professor, Ben-Gurion University of the Negev, Beer Sheva, Israel	2009-date

MEMBERSHIPS ON RELEVANT ADVISORY COMMITTEES

Associate Editor, Journal of Immunology	1998-1999
Section Editor, Journal of Immunology	1999-2002
NIH Immunobiology (IMB)/Cell and Molecular Immunology-B (CMI-B) Study Section, ad hoc	2000-
Intramural Reviewer, Experimental Immunology Laboratory, NCI-Frederick	2006, 2010
NIH Innate Immunity and Inflammation (III) Study Section, standing member	2013-2017

C. SELECTED PEER-REVIEWED PUBLICATIONS

1. Campbell, K.S., Cambier, J.C. B lymphocyte antigen receptors (mIg) are non-covalently associated with a disulfide-linked, inducibly phosphorylated glycoprotein complex. EMBO J. 2:441-448, 1990.
2. Campbell, K.S., Hager, E.J., Friedrich, R.J., Cambier, J.C. IgM antigen receptor complex contains phosphoprotein products of B29 and mb-1 genes. Proc. Natl. Acad. Sci. U.S.A. 88:3982-3986, 1991.
3. Campbell, K.S., Dessing, M., Cella, M., Colonna, M. Tyrosine phosphorylation of a human killer inhibitory receptor recruits protein tyrosine phosphatase 1C. J. Exp. Med. 184:93-100, 1996.
4. Yusa, S.I., Campbell, K.S. SHP-2 can play a direct role in the inhibitory function of killer cell Ig-like receptors in human NK cells. J. Immunol., 170:4539-4547, 2003.
5. Kikuchi-Maki, A., Catina, T.L., Campbell, K.S. Cutting Edge: KIR2DL4 transduces signals into human NK cells through association with Fc receptor γ protein. J. Immunol. 174:3859-3863, 2005.
6. Alvarez Arias, D.A., Campbell, K.S. Protein kinase C regulates expression and function of inhibitory killer cell Ig-like receptors in NK cells. J. Immunol. 179:5281-5290, 2007.
7. Miah, S.M., Hughes, T.L., Campbell, K.S. KIR2DL4 differentially signals downstream functions in human NK cells through distinct structural modules. J. Immunol. 180:2922-2932, 2008.
8. MacFarlane IV, A.W., Yamazaki, T., Fang, M., Sigal, L.J., Kurosaki, T., Campbell, K.S. Enhanced NK cell development and function in BCAP-deficient mice. Blood 112:131-140, 2008.
9. Purdy, A.K. Campbell, K.S. SHP-2 expression negatively regulates NK cell function. J. Immunol. 183:7234-7243, 2009.
10. Miah, S.M., Purdy, A.K., Rodin, N.B., MacFarlane IV, A.W., Oshinsky, J., Alvarez-Arias, D.A., Campbell, K.S. Ubiquitylation of an internalized killer cell Ig-like receptor by Triad3A disrupts sustained NF-κB signaling. J. Immunol. 186:2959-2969, 2011.
11. Ni, M., MacFarlane, IV, A.W., Toft, M., Lowell, C.A., Campbell, K.S., Hamerman, J.A. B-cell adaptor for PI3K (BCAP) negatively regulates Toll-like receptor signaling through activation of PI3K. Proc. Natl. Acad. Sci. U.S.A 109:267-272, 2012.
12. Grier, J.T., Forbes, L.R., Monaco-Shawver, L., Oshinsky, J., Atkinson, T.P., Moody, C., Pandey, R., Campbell, K.S., Orange, J.S. Human immunodeficiency-causing mutation defines CD16 in spontaneous NK cell cytotoxicity. J. Clin. Invest. 122:3769-3780, 2012.
13. Campbell, K.S., and Hasegawa, J. NK cell biology: an update and future directions. J. Allergy Clin. Immunol., 132:536-544, 2013.
14. Brusilovsky, M., Cordoba, M., Rosental, B., Hershkovitz, O., Andrake, M.D., Pecherskaya, A., Einarson, M.B., Zhou, Y., Braiman, A., Campbell, K.S.*, and Porgador, A.* Genome-wide siRNA screen reveals a new cellular partner of NK cell receptor KIR2DL4: heparan sulfate directly modulates KIR2DL4-mediated responses. J. Immunol., 191:5256-5267, 2013. *Corresponding authors.
15. MacFarlane 4th, A.W., Jillab, M., Plimack, E.R., Hudes, G.R., Uzzo, R.G., Litwin, S., Dulaimi, E., Al-Saleem, T., and Campbell, K.S. PD-1 expression on peripheral blood cells increases with stage in renal cell carcinoma patients and is rapidly reduced after surgical tumor resection. Cancer Immunol. Res., 2:320-331, 2014.