**Editorial comments:**  
Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Response: The entire manuscript has been carefully re-read and numerous grammatical errors have been addressed.

2. Figure 1: Please describe panels A and B.

Response: Thank you for pointing out the fact we forgot to update the figure legend of Figure 1. The figure legend has now been updated to describe panels A and B.

3. Figure 2B: Are the lines cut off at 10-22 s?

Response: Yes, but this is for a very good reason. This the time in which the sample is taken off the flow cytometer in order to add the antigen. Although the instrument continues to acquire data during this time, the values at this point in time are confusing and meaningless, therefore, it is standard practice to not show data during this time. To avoid confusion for the reader, we have added a note about this in the figure legend: *“Note: between 10-22 s is when the stimulation is added and, therefore, no data is acquired during this time.”*

4. Figure 5: Please line up the panels better.

Response: Thank you for the good suggestion. We have now aligned panel A and B horizontally, and A and C vertically.

5. Summary: Please shorten the summary to meet the 10-50 word limit.

Response: The summary has been shortened to 50 words.

6. Please define all abbreviations before use (DMSO, RT, DTT, etc.).

Response: Abbreviations have been defined.

7. Please use SI abbreviations for all units: L, mL, µL, h, min, s, etc.

Response: SI abbreviations have been used and defined.

8. Please include a space between all numbers and their corresponding units: 15 mL, 37 °C, 60 s; etc.

Response: The requested changes have been made.

9. Please convert centrifuge speeds to centrifugal force (x g) instead of revolutions per minute (rpm).

Response: The requested changes have been made.

10. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

Response: The requested changes have been made.

11. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents.  
For example: Jackson Labs, stock number 000651, Sephadex G50, Kimwipe, Avanti, NuPAGE, Coomassie, etc.

Response: The requested changes have been made.

12. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc. Please revise 1.3, 1.6, 4.12, etc.

Response: Sentences have been changes to make more imperative whenever possible and several ‘Notes’ have been added.

13. The Protocol should be made up almost entirely of discrete steps without large paragraphs of text between sections. Please simplify the Protocol so that individual steps contain only 2-3 actions per step and a maximum of 4 sentences per step. Please revise 1.3, 1.6, 1.17, 4.13, etc.  
14. There is a 10 page limit for the Protocol. Please revise the protocol section to meet this page limit.

Response: The suggested sections have been shortened as requested and the revised protocol section now meets the 10-page limit.

15. There is a 2.75 page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

Response: The filmable content, comprising a total of 2.75 pages, has been highlighted in yellow in the revised manuscript.

16. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.

Response: We have taken significant care to provide a contiguous and logical narrative and that sentences are complete and written in the imperative tense.

17. References: Please do not abbreviate journal titles.

Response: All journal titles have been spelled out in full.

**Reviewers' comments:**  
  
**Reviewer #1:**   
Manuscript Summary:  
The manuscripts by Bednar et al. describes a method of peanut allergy in mice that is adoptively transferred by spleen from peanut-sensitized mice, it is reproducible and minimize the use of adjuvants and animals. To achieve an increase in -Ara h 2 specific IgE and IgG1 antibodies the animals are boosted with liposomal nanoparticles containing Ara h 2 allergen.  
Taking into account that food allergy models are rare, the proposed model is promising. However there are some questions that should be addressed.

Response: Thank you for the positive feedback on the promise of this new food allergy model.  
  
Major Concerns:  
1. Regarding booster: There is no information whether other types of booster would give similar results. For instance, the authors only included a booster with liposomes containing Ara h 2. They should include booster with Ara h 2 without liposomes as well as with peanut extract in order to validate the efficacy of Ara h 2 vs extract or liposome with Ara h 2 vs liposome with peanut extract.

Response: We agree with the reviewer that all the possible controls for different forms of the antigen during the boosting step (one day after the adoptive transfer) would be nice show. Since writing the first version of this manuscript nearly 6 months ago, we have been moving the model into B6 mice and have indeed performed all these controls. We will be including this data in a subsequent publication but would be happy to provide the data to satisfy the reviewer if required. What our data shows that indeed liposomal delivery of antigen is absolutely required to induce the allergic response. While soluble allergen does induce low levels of antibody, these are not enough to drive an anaphylactic response upon a subsequent challenge. These results are in line with many other studies demonstrating that the multivalent display of antigen on liposomes can potently stimulate B-cell responses and that liposomal nanoparticles have the added benefit that they can effectively prime T-cell responses through being taken up non-specifically by APCs. With these things in mind, it is important to point out that the primary objective of this manuscript is to demonstrate the basic techniques of preparing antigenic liposomal nanoparticles, their testing in B-cell stimulation assays using calcium flux assay, and the steps involved in setting up and measuring an anaphylactic response in an allergic mouse model.

2. IgE production: ELISA for specific IgE usually is not performed directly since specific IgG1 antibodies that are in higher concentrations than IgE antibodies could compete with epitopes recognized by IgE. For instance, to measure specific IgE in OVA model. The serum IgE is first captured by anti-IgE antibody and then is revealed by biotinylated OVA.

Response: We understand the reviewer’s point and are aware of possible interference by IgG in our ELISA, however, we are clearly able to detect Ara h 2-IgE using our methodology. Additionally, we have recently published some of our work using the more traditional peanut allergy model in the Journal of Allergy and Clinical Immunology (Orgel, et al., JACI, 2017, Exploiting CD22 on antigen-specific B cells to prevent allergy to the major peanut allergen Ara h 2) and other well-known researchers in the field, such as Hugh Sampson at Mount Sinai, also use the same method as our laboratory (see for example, Srivastava, et al. Journal of Allergy and Clinical Immunology, 2016, Investigation of peanut oral immunotherapy with CpG/peanut nanoparticles in a murine model of peanut allergy). Therefore, we feel that our method is well-established and is an acceptable technique to measure allergen-specific IgE in mouse serum.

**Reviewer #2:**  
Manuscript Summary:  
This paper by Bednar et al. describes a method of inducing allergen specific IgE responses, in particular to the peanut protein Ara h2, using a mouse model. In this model, mice are sensitized to whole peanut in a classical adjuvant based technique. Spleneocytes and then transferred to naive mice and then IgE to Ara h2 is boosted with injection of a liposome conjugated Ara h2 and then followed by a free Ara h2 injection. This increased both IgE and IgG responses to Ara h2. The protocol is generally well written and clear and should be published. This technique certainly has merit and is an interesting use of liposomes, but the authors need to reevaluate their claims of both simplicity and reproducibility.

Response: Thank you for the positive feedback that this should be published. Thank you for the extremely helpful comments and suggestions that have improved the manuscript.

Major Concerns:  
1. The data is not sufficiently convincing to claim that this method is highly reproducible. They only had 4 mice per group and the results were fairly variable, which is to be expected in mouse experiments. They do not show a side by side comparison with other methods of inducing peanut allergies. They should have at least taken mice who were sensitized to peanut without transferring the splenocytes and used those as a control, not mice whose splenocytes were transferred and then challenged with PBS.

Response: Although the data in the manuscript only includes four mice per group, we have repeated this experiment several times and seen very reproducible results. For example, the average body temperature drop 30 minutes after i.p. challenge with Ara h 2 for two independent experiments were 7.2 ± 1.1°C and 6.7 ± 1.9 °C (n=4 each). These standard deviations are tighter and stronger than what we typically see for peanut sensitized mice that are i.p. challenged with peanut, 3.1 ± 2.0 °C (n=12). The goal of this manuscript is to present this new model, and show visually how we challenge and monitor anaphylaxis, not to provide a direct comparison to other peanut allergy models.

2. If they claim that there is an increase in Ara h2 specific antibodies in these mice (Figure 5A), it is critical that they also show IgE and IgG levels of other Ara h proteins, in particular Ara h 1 and Ara h 6 for comparison to show that their technique is increasing Ara h 2 specific antibodies.

Response: In response to this comment, we have quantified Ara h 1-specific IgE and IgG1, and we see an increase in both after the Ara h 2 boost injection. This is consistent with data shown in our previous publication (Orgel, *et al*., JACI, 2017) and another publication showing human IgE cross-reactivity between Ara h 1, 2 and 3 (Bublin, *et al*. JACI, 2013). Since there is known cross-reactivity between these peanut allergens, we are not surprised by these findings. Our data do show an increase in Ara h 2-specific IgG1 and IgE and these correspond to anaphylactic reactions observed with Ara h 2 challenge in the mice.

3. They need to do a better job in the intro stressing what separates this method from other methods. Does it provide more Ara h2 specific antibodies? Is this method more simple? Given the need for multiple injections and transfer of splenocytes, I would certainly say this method is not more simple than other adjuvant based methods.

Response: We acknowledge that this model is not necessarily ‘simpler’ than other models and do not believe that we have used language to describe it in these terms. Having said this, we do think there is room to improve the description of the advantages of this method over previous methods. To address this, we have added the following text to the discussion:

“Compared to these conventional approaches, this adoptive transfer model offers three primary advantages. The first is that it enables memory B- and T-cells specific to the allergen to be studied more directly. Indeed, evidence now strongly implicates memory B-cells as the major source of long-term allergies18. Second, because the same number of memory B- and T-cells are implanted into each recipient mouse, the allergic responses are minimally variable. Third, this model provides the opportunity to study allergic responses to individual allergens, which is useful in the context of testing new immunotherapies.”

4. One interesting aspect of this work that is not sufficiently discussed is the potential for adoptive transfer of human splenocytes into a mouse model. It is well known that one of the significant challenges in mouse models of allergies is that mice IgE bind to different epitopes on allergens than humans. If T cells and B cells from sensitive individuals are taken and transplanted into a immunodeficient mice, this technique could be used to sensitize mice with the appropriate epitope profiles.

Response: This is a very intriguing point that we had also contemplated but felt that it might not be appropriate because of the lack of appropriate cells (e.g. mast cells) in such mouse models. However, upon reading more about this subject, we agree with the reviewer that this would be appropriate to discuss as an application of the model. As such, we have added the following text to the discussion:

“One intriguing application of this model is to use the general approach in the context of a humanized mouse model, where allergic responses have been studied in immunized models21. Applied in the context of an adoptive transfer model, a humanized mouse model could be a powerful approach to expanding and *in vivo* testing of B- and T-cells from allergic patients.”  
  
Minor Concerns:  
Some minor grammatical errors, check line 699.

Response: Thank you for pointing out the grammatical error on line 699. The manuscript has been re-read carefully and a number other errors have been fixed.