**Editorial comments:**  
Changes to be made by the Author(s) regarding the written manuscript:  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

The whole manuscript was checked to avoid spelling and grammatical errors.

2. Please provide an institutional email address for each author.

The email addresses which were used institutionally by the authors were given in the first-time of paper submission to JoVE. Please find them at the submission system of JoVE.

3. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. 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. You may use the generic term followed by “(see table of materials)” to draw the readers’ attention to specific commercial names. Examples of commercial sounding language in your manuscript are: Vital River Laboratory Animal Technology, RADSOURCE, Vicryl, Jiangnan Novel Optics, RWD Life Science, Cyagen Biosciences, Gibco, Thermo Fisher Scientific, Agilent, Eppendorf, etc.

Table of Materials and Reagents was setup according to the guideline. Please find the file named Table3. Commercial language was removed from the main text.  
4. Please move the ethics statement before your numbered protocol steps, indicating that the protocol follows the animal care guidelines of your institution.

It is done.   
5. Please adjust the numbering of the Protocol to follow the JoVE Instructions for Authors. For example, 1 should be followed by 1.1 and then 1.1.1 and 1.1.2 if necessary. Please refrain from using bullets, dashes, or indentations.

It is done.  
6. Please revise the protocol to contain only action items that direct the reader to do something (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.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible.

It is done.  
7. Lines 93-102: 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.

It is done.  
8. Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action. See examples below:

Lines 106-107: Is this step for anesthetization? Please mention how proper anesthetization is confirmed.

Necessary revision is made.   
Lines 109-113: Please specify all surgical instruments used. Please split into two steps.

It is done.  
Lines 147, 158, etc.: Please specify the surgical instrument used throughout the protocol.

It is done.  
Lines 154-155: This step is unclear. Do you mean that perform anesthesia, skin prepping, and separation of abdominal aorta for the healthy rat? Please specify the numbered steps that are repeated here.

It is done.  
Lines 197-198: Please add more details. For instance, for how long are the specimens fixed with 10% formalin? How to perform paraffin embedding, and thin sectioning? What is the thickness of the sections?

Necessary revision is made.  
Line 206: How are the intimal thickness and full thickness measured?

Necessary revision is made.  
Line 219: Do you mean liquid nitrogen?

Yes, necessary revision is made accordingly.  
Line 267: Please provide composition of lysis buffer A. If it is purchased, please cite the Table of Materials.

Yes, it was purchased. More information is provided in JOVE\_Materials.   
9. Lines 116-121: Please split into two steps.

Necessary revision is made.  
10. Lines 129-140: The Protocol should contain only action items that direct the reader to do something. Please move the equipment information to the Materials Table.

Necessary revision is made.  
11. Lines 296-298: Please write the text in the imperative tense.

Necessary revision is made.  
12. Please combine some of the shorter Protocol steps so that individual steps contain 2-3 actions and maximum of 4 sentences per step.

It is done.   
13. Please include single-line spaces between all paragraphs, headings, steps, etc.

It is done.  
14. After you have made all the recommended changes to your protocol (listed above), 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.

It is done. The part with grey background include the essential steps of protocol for video recording.   
15. 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. Please do not highlight any steps describing anesthetization and euthanasia.

It is done.  
16. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

It is done.  
17. Table 1 is missing from the submission. Please upload Table 1 to your Editorial Manager account as an .xls or .xlsx file.

Table1 was uploaded to the submission system at the beginning. But I do not know why it was not presented to you. Anyway, I do it again. Please find attached xlsx file.  
18. Figures 2-4: Please define error bars in the figure legend.

It is done.  
19. Figures 3 and 4: Please briefly describe different panels of the figure.

It is done.   
20. Figure 4: Please define scale bars in the figure legend.

It is done.  
21. Discussion: Please discuss critical steps within the protocol.

It has been discussed in the paragraphs from line 428 to 450.  
22. References: Please do not abbreviate journal titles.

Full journal title is provided.   
  
**Reviewers' comments:**  
  
**Reviewer #1:**  
Manuscript Summary:  
This study investigated the homing of MSCs in the model of irradiation vasculitis after their intravenous infusion. Thus six study groups were employed including that of irradiation plus MSC infusion and irradiation then transplantation of irradiated aorta to a healthy recipient rat followed by MSC infusion, together with other needed control groups. The study results showed that homing of MSCs was significantly higher in the transplanted irradiated aorta and thus showed better histological repair and reversed inflammatory response.  
  
Major Concerns:  
1- Table 1 is not included in the manuscript which made it hard to assume the study groups , the reviewer assumed such information from the given figures. Table 1 should be added and reviewed

Table1 was uploaded to the submission system at the beginning. But I do not know why it was not presented to you. Anyway, I do it again. Please find attached xlsx file.

2- Some details are missing in the MSC infusion protocol , these details are mandatory for others to follow your protocol of work and they include :

-- DMEM basal media used , was it high or low glucose , with or without L- glutamine

Low-glucose DMEM with L-glutamin was used in the study.

-- The passage of MSC cells used for the infusion

Passage 3 of MSC cells after viral transfection was used for intravenous infusion.

-- What MSCs characterized method was used before the infusion of these cells

The expression of MSC markers was checked by realtime-PCR. As a result, there were no significant changes in the expression of positive MSC makers like CD90, CD44, CD29, and negative markers CD34, CD45, CD11b/c. The relevant data are demonstrated in Table 2.

-- Duration of infusion of cells through the tail vein

The single dose of 2 × 106 cells was prepared in 0.4ml serum-free medium and infused to each rat via tail vein for 15 sec.   
  
Minor Concerns:  
1- Authors should mention in animals section that male rats were used for bone marrow isolation and transfusion in the female rats, for the purpose of tracking the cells in the transplanted aorta using the SYR gene as its first mention is in the results section

The changes are made according to the suggestion.

2- It is clear that the aim of this study is evaluating the MSC homing for irradiation vasculitis thus irradiated vessels were sequestered from adjacent tissue and transplanted in recipient animal representing the RT- plus- Tx model. Showing the promising study results, how do the authors think such model could be translated clinically so that avoid scattering of transplanted cells to adjacent tissue?

Our study was based on the previous research showing that local irradiation enhanced MSC recruitment to the field of radiation. Sequestration of irradiated vessels from adjacent tissue was an effective way to make it sure that radiation energy was exclusively delivered to target vessels. As a result, MSC recruitment was improved by our attempt. Our study provided further evidence to the previous research and suggested the advantage of local irradiation. Based on the theory, many methods of delivering local irradiation like intensity modulated radiation therapy are recommended not only to reduce the risk of injury to unintentionally irradiated organs but to improve the effect of MSC-based therapy.   
  
**Reviewer #2:**  
Manuscript Summary:  
The authors have developed a model of recapitulates aspects of radiotherapy induced vasculitis. In an effort to limit confounding factors, the authors have developed a model in which the abdominal aorta of a donor rat is extracted and subsequently irradiated prior to transplantation into a recipient rat. Following transplantation the animals are treated with syngenetic, GFP labeled MCSs. Animals are then euthanized at Day 90 (60 days post initial treatment administration) for histological analysis. This is an interesting method that models key aspects of radiotherapy induced soft tissue damage that allows for the investigation of both MSC homing and MSCs' ability to modulate RT-induced tissue remodeling.  
  
Major Concerns:  
None  
  
Minor Concerns:  
The rationale for the use of a non-parametric rank test statistical analysis (Mann-Whitney) is not clear to this reviewer, as there the samples were all garnered from a similar system so there would be an expectation that their respective distribution would be the same, in addition to the same samples sizes were used for each treatment group.

Although the method of data collecting for each variable was same between study groups, the variable did not necessarily obey normal distribution which was considered as the criteria of parameter analysis. For example, the relative intimal thickness (the percentage of thickness of intima to full vascular wall) and the cell number per high power filed were more likely to obey Poisson distribution than normal distribution. In that case, Mann-Whitney test was more suitable than parameter analysis to be used in our data analysis.