Reviewer reports:

Reviewer #1:

In this manuscript the authors reported that CSE/H2S inhibited ferroptosis of HUCMSCs. However, the in vivo data is confusing. Major concerns 1.In Fig3, can the authors explain how HUCMSCs+Fer-1 was better than HUCMSCs alone? 2.Does HUCMSCs treatment for pulmonary arterial hypertension induce ferroptosis? If not, what is the point to administration of HUCMSCs with Fer-1? Minor concerns 1.Language mistakes, such as "hypoxia-induce mice", "Ferroptosis is characterized by iron-dependent lipid peroxidation co-exists with oxidative stress", "the upregulation CSE/H2S pathway" et al. The full name and the abbreviation appear repeatedly in the manuscript. For example, "human umbilical cord MSCs (HUCMSCs)".

To Reviewer 1:

Thank you for pointing this out.

Question 1. As an inhibitor of ferroptosis, Fer-1 suppressed ferroptosis in HUCMSCs, which improved cell survival after transplantation. Thus, after pre-treated with Fer-1, HUCMSCs improved the treatment effect in PAH mice.

Question 2. In this study, we focused on the ferroptosis in HUCMSCs, not in the PAH-induced ferroptosis. Thus, we will explore the change of ferroptosis in PAH in the future.

Question 3. I have re-polished the English language.

Reviewer #2:

In this research article the Authors aimed at dissecting the role of CSE/H2S pathway in the modulation of ferroptosis, an iron-dependent form of programmed cell death, that may be implicated in low retention and engraftment of mesenchymal stem cells (MSCs) after their in vivo delivery. They placed this issue within the context of a mouse model of hypoxia-induced pulmonary arterial hypertension (PAH) and designed a number of experimental procedures to investigate the role of ferroptosis in human

umbilical cord MSCs (HUCMSCs) dynamics in vitro and following in vivo delivery into PAH mice. The Authors used Erastin and Ferrostatin-1 (Fer-1) to induce or inhibit ferroptosis, respectively. Overexpression or downregulaton of CSE was afforded in HUCMSCs via transfection with specific vectors. Bioluminescence imaging was used to follow HUCMSC post-delivery survival in vivo. Cell viability, iron accumulation, reactive oxygen species (ROS) production, cysteine uptake, and lipid peroxidation were tested in HUCMSCs. Ferroptosis-related proteins and the S-sulfhydration of Keap1 were also investigated. In hypoxia-induced PAH mice, CSE overexpression improved post-delivery HUCMSC survival despite Erastin treatment. In vitro, CSE overexpression improved H2S production and counteracted the pro-ferroptosis effect of Erastin, ameliorating cell viability, decreasing iron level, ROS production, lipid peroxidation. CSE activation/overexpression decreased lipid peroxidation, improving structural mitochondrial features, ultimately tending to restore the physiological expression patterning of a number of ferroptosis-related proteins. In vivo, CSE activation/overexpression proved effective in reversing pulmonary arterial wall remodeling, ameliorating the profile of right ventricular arterial pressure. Overall, this is an interesting study, based upon a thorough experimental approach, with appreciable novelty and potential biomedical implication. Unfortunately, the same study presents a number of critical issues that require a major revision and preclude consideration in the present form.

In detail: - There are frequent grammar errors, misspelling, single/plural errors in verbs. As a result, the English language requires considerable degree of polishing. –

In the section "CSE inhibition exacerbated Fer-1-suppressed ferroptosis in HUCMSCs". Pages 21-22, "In Fig. 4, cell apoptosis, the level of Fe2+, ROS, lipid peroxidation and MDA, and the expression of 4-HNE, TFRC1, FTH1 and NOCA4 were significantly increased. Indeed, FTH1 was decreased, compared to control! This must be corrected and an explanation for the differential

patterning should be anticipated in this section, and further elaborated in the Discussion Section. –

Still in the section "CSE inhibition exacerbated Fer-1-suppressed ferroptosis in HUCMSCs", The pattern of GPX4 is not reported, nor discussed, while a GPX4 pattern is reported in panel M of Fig. 4. - Similarly, in the section "CSE overexpression negatively regulates Erastin-induced ferroptosis in HUCMSCs", the effect of CSE overexpression on GPX4 is not reported, nor commented.

- In the same section, "As shown in Fig. 5, there were a significant decreased cell apoptosis, the level of Fe2+, ROS, lipid peroxidation and MDA, and the expression of 4-HNE, TFRC1, FTH1 and NOCA4". Indeed, CSE overexpression, while decreasing the expression of 4-HNE, TFRC1, and NOCA4, conversely increased FTH1 expression, as well as GPX4 expression. Moreover, in the same section, CSE overexpression-mediated increase in SCL7A11 expression is not reported, nor commented, while it's clearly evident in panel M of Fig. 5.

- Importantly, in Fig. 5 the effect of CSE overexpression alone, in the absence of Erastin is missing (only Erastin + CSE is shown). Missing this data is an important failure.

- Overall, in this section the differential effects elicited by CSE overexpression alone or in combination with Erastin, decreasing 4-HNE, TFRC1, FTH1 and NOCA4, while enhancing FTH1, GPX4, and SCL7A11 expression is totally missing!

- In the same above section, another important missing point is the lack of reporting the effect of Brusatol. Its inhibitory effect is shown in Fig 5, in panels A, B, C, D, E, F, G, H, I, J, K, L, and M. Nevertheless, in this part of the Results section the same effect is not reported! The only part of the results section describing the effect of Brusatol is in the section "CSE/H2S pathway induced Keap1 S-Sulfhydration and Nuclear Translocation of Nrf2" dedicated to Fig. 6. Even then, there is no indication of why it was used. This is important for readers who may not necessarily be specialists in the field. The Authors should

have been briefly introducing the rationale for cell exposure to Brusatol in the Results section, where it was more appropriate, that is the description of results from Fig. 5. Then the effects could have been described and commented in detail in the Discussion Section.

- In the section "CSE/H2S pathway induced Keap1 S-Sulfhydration and Nuclear Translocation of Nrf2" The results obtained in the presence of different combinations of DTT with other agents should have been described more in detail. In particular, the effect of DTT on nuclear translocation of Nrf2 must be detailed. In this section it is reported: "DTT reversed the Kepa1 (should be Keap!) S-sulfhydration and reduced Nrf2 protein expression in the nucleus". In panel F of Fig. 6 it is evident that Erastin alone reduced the nuclear levels and likely translocation of Nrf2, which is the opposite pattern as compared in control conditions (Panel F, Fig. 6: higher nuclear than cytoplasmic levels of Nrf2). In panel H, Erastin alone does the opposite of what's shown in panel F, that is it enhances the nuclear level of Nrf2. This discrepancy must be explained!

- In Fig. 6, Erastin + CSE enhances the nuclear level of Nrf2 (pane F), but DTT, in the presence of Erastin + CSE elicited the opposite (a cytoplasmic increase and a nuclear decrease). The reasons for such DTT effect should be anticipated in the Results section and then further addressed in the Discussion Section. Again, the effect of CSE overexpression alone is missing. –

The Discussion section is highly problematic. In its first part, the characteristics and the roles of system Xc-, GPX4, and other players discussed in the central/last part of this section should be briefly outlined to give the reader an easier way to follow the discussion of the experimental observations. - The differential patterns showing a decrease 4-HNE, TFRC1, FTH1 and NOCA4, and an increase in FTH1, GPX4, and SCL7A11expression following CSE overexpression are not discussed on mechanistic bases in the Discussion Section. Again, there is no synthetic presentation of the relevance of each of these individual players within the investigated context. Some of these players are generically acknowledged as products of antioxidant genes "Nrf2 is one of

the major cellular defense lines against ferroptosis through promoting antioxidant genes translation" (page 27, Discussion Section). (This should be written as: Nrf2 is one of the major cellular defense lines against ferroptosis by promoting antioxidant gene transcription).

- Another major point is the real lack of discussion of the effects elicited by DTT on nuclear translocation of Nrf2 in the absence and presence of Erastin and CSE overexpression. In other words, a consistent part of the data reported in Fig.6 are not commented in the Results section, nor they are really explained in the Discussion section. - On the whole, the discussion section is very "fragmented", lacking an accurate discussion of the experimental findings. In conclusion, while the Authors have produced an important body of potentially relevant observations, the whole study requires substantial rewriting in the Discussion section, and addressing of the criticisms highlighted above.

To Reviewer 2:

Thank you for pointing this out. I apologized for my negligence. I have re-written these paragraphs, re-polished the English language, and re-layout Fig. 6.

Reviewer #3:

This manuscript focused on the influence of the CSE/H2S pathway on ferroptosis in human umbilical cord MSCs remains unclear. The model suggested that pregulation of the CSE/H2S pathway induced the S-sulfhydration of Keap1, which contributed to the inhibition of ferroptosis. The influence of the CSE/H2S pathway on ferroptosis in human umbilical cord mesenchymal stem cells (MSCs) remains a topic of ongoing research, and there may not be a conclusive answer available at the moment. However, This study suggests that these findings could open up a novel therapeutic approach to enhance the protective capacity of transplanted MSCs in the context of PAH. This study suggests that there is evidence indicating that inhibiting ferroptosis improves the survival of HUCMSCs transplants in mice with hypoxia-induced

PAH. This is an interesting finding in the context of regenerative medicine and PAH research. Ferroptosis is a form of regulated cell death, and the study suggests that by inhibiting this process, the survival of HUCMSCs transplants in a PAH model is enhanced. Also, the manuscript required editing to address grammar or style errors. Some text is repetitive and there tends to be a bit of Discussion creeping into the Results. A very large amount of work was involved in the study, and as far as I can determine, the work is solid. The results are not always new or interesting. Notwithstanding, ferroptosis is a form of regulated cell death characterized by iron-dependent lipid peroxidation. It is an emerging area of research in the field of cell biology, and understanding the molecular mechanisms underlying ferroptosis in different cell types, including HUCMSCs, is of great interest. The reviewer declared no conflict of interest.

To Reviewer 3:

Thank you for the review. I have re-written part of this manuscript and re-polished the English language.