REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

They show that MHC-match-donor cells can reduce inflammation, leading to an increased survival of TH-positive neurons. These data are presented in a quantitative manner and now I believe the manuscript represents a significant advancement in stem cell therapy, except one point as for SFig6e.

The pictures they added do not support the author’s conclusion. The author claimed that "no GFP+ grafted cells expressed Iba-1 or CD45 in vivo (Supplementary Fig. 6c, e)". However, the pictures they provided showed that some GFP+ cells look like Iba-1 positive. Therefore it is difficult to believe author’s claim based on their picture. How did the authors define Iba-1 signals? Are the red signals in the picture are mostly background? Could the authors provide the quantification method of Iba1 with quantitative data?

7. Iba-1+ / GFP+/- picture was not shown although the authors mentioned this in the text. Please add this photo.
→ We added pictures of immunohistochemistry (GFP/Iba-1/DAPI) as Supplementary Fig. 6e and changed the legend accordingly.
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→We apologize for providing poor quality pictures of GFP/Iba-1 immunostaining. The poor quality was due to low specificity of the GFP antibody that we used previously. For the revised manuscript, we changed the GFP antibody (GFP: rat IgG, Nakalai 04404-84, clone GF090R, 1:1000) and stained the slices again. We noticed that the original GFP antibody not only stained GFP poorly, but also affected the Iba-1 staining negatively. The combination of the new GFP antibody and the same Iba-1 antibody improved the Iba-1 signal.

To quantify Iba-1 positive cells, we counted the slices of single-immunostaining with DAB-Ni enhancement (Fig.6 a, b, d).
Previous version of Supplementary Fig. 6e (GFP; green, Iba-1; red, DAPI; blue)

New version of Fig. 6i (GFP; green, Iba-1; magenta, DAPI; blue)
We changed the color of the Iba-1 staining from red to magenta according to the editor’s request.

As a reference, a picture of low magnified view is shown below.

GFP Iba-1 (low magnified view of Fig. 6 i)

As we showed in Fig. 2 c, d and Supplementary Fig. 1 of the newest submission, the efficiency of GFP labeling for donor cells was not high (20% on differentiation day14).

Recent lineage tracing studies have shown that microglia originate from yolk sac erythromyeloid progenitors (EMP) generated during primitive hematopoiesis (Ginhoux et al., 2010; Kierdorf et al., 2013; Schulz et al., 2012). This year, several groups have reported protocols that induce microglia from human iPSCs. In those protocols, cells of hematopoietic lineage (CD34+, CD43+, etc) were firstly induced from iPSCs (Pandya et al., 2017; Abud et al., 2017; Douvaras et al., 2017).

In our present study, more than 99% of donor cells were positive for PSA-NCAM, indicating the cells were of neuroectodermal lineage.

Taking the above into consideration, we conclude that Iba+1+ microglia in the grafted area were derived from the host brain and not from the grafted cells.

References


