Response to Notter and Meyer's Letter to the Editor regarding:

Increased expression of Translocator protein (TSPO) marks pro-inflammatory microglia but does not predict neurodegeneration

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Short title: TSPO in pro-inflammatory non-neurodegenerative microglia

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Dear Editor

We appreciate the praise by Drs. Notter and Meyer on the relevance of our study regarding the improved characterization of TSPO upregulation in neuroinflammatory conditions and we thank them for pointing out an error in one of the figures.

While performing immunofluorescent staining for TSPO in the *Mfp2* knockout mouse brain, we noticed that all microglia were TSPO positive (illustrated in Fig. 3i) and that only few TSPO positive cells did not stain for the microglial marker Iba1 in different brain areas. When visualizing reactive astrocytes using GFAP antibodies, only few expressed TSPO. Drs Notter and Meyer rightly noticed that figure 3k in the manuscript is not representative for our findings described in the text. The figure also does not reflect the widespread astrogliosis in the *Mfp2*^{-/-} brain that we previously reported¹. We have carefully rechecked staining of brain slices and found that an inadvertent error was introduced in fig 3k during one of the revision steps of the manuscript. We have performed additional experiments with new staining reagents to accurately detect GFAP (1:200 mouse aGFAP (Sigma)-1:200 goat anti mouse Alexa568). A relatively large number of reactive astrocytes are found in the *Mfp2*^{-/-} brain in agreement with our earlier findings¹ and only few reactive astrocytes also express TSPO.

These results are in agreement with the phrasing in our manuscript “increased TSPO levels are mainly attributed to reactive microglia in the *Mfp2*^{-/-} brain”.

*In the cortex of wild type mice, TSPO and GFAP levels are very low. In *Mfp2*^{-/-} cortex, both TSPO and GFAP immunoreactivity are markedly increased but only few GFAP+ astrocytes express TSPO (arrows). This is in accordance with Fig3i in the manuscript in which TSPO colocalizes with nearly all Iba1+ microglia. Separate channels for GFAP and TSPO are shown. In the merged picture also nuclear DAPI staining is visualized. Similar data were obtained in other brain regions (brainstem, colliculus, thalamus).*

We thank the editor for giving the opportunity to make corrections. We are grateful to Dr. Ritesh Kumar Baboota (Laboratory for Cell Metabolism, KU Leuven) for performing stainings.

- 1 Huyghe, S. *et al.* Peroxisomal multifunctional protein-2 deficiency causes motor deficits and glial lesions in the adult central nervous system. *Am J Pathol* **168**, 1321-1334 (2006).