point-by-point response

Reviewer #1

1. In the abstract you refer to a hypothesis without actually spelling out the hypothesis.

Response: We have corrected that sentence to make clear that the aim of this study was to verify the finding reported by Pickrell et al., 2015 (see abstract, methods, page 2)

1. The reference for the Padel mice should be inserted when the model is mentioned in the introduction.

Response: We agree with the reviewer’s comment and added additional references for the Padel mice (see page 3, 4, 10).

1. The animal section is incomplete. The strain number for the mutator mice from JAX must be included. It would be helpful to have information about age and sex in the animal section as well.

Response: We agree, and have added additional information about mutator mice including their sex in that section (see page 4). The age of mice from our studies were already described in the methods or in the results.

1. The abbreviation LM needs to be defined on the first instance that it is used.

Response: We have added that abbreviation in the text (see page 5).

1. You were aware that sliding down the pole during the pole test has already been reported. Have you collected data to validate/quantify this in your experiments? This would be a very useful additional piece of information.

Response: Yes, we always create notes during our behavior experiments and have added the additional data of the mice’s slide-down rates of WT, Padel, MT and MT/Padel mice and their statistical comparison on the page 8 to show that MT and MT/Padel mice exhibited similar higher percentage of trials in which mice slid down the pole during the pole test. This effect was not dependent on the parkin mutation.

1. The speculation about the GFP/Neo genes resulting in DA neuron loss needs more refinement and other studies in support of such a mechanism need to be cited.

Response: We have added additional refinement and a references to the text on the page 8 and 11.

1. There are multiple issues with orthography and grammar that need to be reviewed.

Response: We have revised the whole manuscript and corrected all grammar mistakes we have found.

Reviewer #2

1. Abstract: The author should clearly state their hypothesis in the last sentence of the background. When I read the Methods section ("We tested this hypothesis by .....") in the abstract, I was still wondering what their hypothesis was.

Response: We have corrected that sentence to make clear that the aim of this study was to verify the finding of Pickrell et al., 2015 (see abstract, methods, page 2)

1. The authors should provide more details about the statistical analysis performed.

Response: All statistical analyses were performed by using H- or u-test, which are described in the figure legends. We have added this information to the methods section (see Page 5)

1. I do not think only counting the numbers of TH-positive neurons is a good method to measure neurodegeneration. TUNEL staining and Fluoro-Jade B staining should be performed to measure neuronal apoptosis and neurodegeneration.

Response: TUNEL and Fluoro-Jade staining have been shown their ability to mark dying neurons in some particular acute neurodegenerative mouse models. While the TUNEL staining marks apoptosis, the staining mechanism of Fluoro-Jade in such neurons is still not known. We do not think that both methods are suitable for measuring neuronal loss and neurodegeneration related to Parkinson’s disease because of the following reasons:

1. Degeneration of DA-neurons in Parkinson’s disease is a long, slow and chronic process so that it is difficult to capture enough dying neurons at a particular time point. Therefore, the TUNEL and Fluoro-Jade staining were not often used for Parkinson research due to their lacking capability to visualize its disease progression.
2. The exact mechanism of neurodegeneration of DA-neurons in Parkinson is still elusive and the role of apoptosis of DA neurodegeneration in PD is highly controversial. The TUNEL (apoptosis) and Fluoro-Jade staining (with its unknown staining mechanism) may not be able to mark those dying DA-neurons in Parkinson. To our knowledge, these methods are not suitable for characterization of Parkinson pathogenesis.
3. It is well known that one-year old WT and Padel mice do not exhibit DA-neurodegeneration. The comparison of total DA-neurons in SNpc at the end of their expected lifespan of one year old for MT and MT/Padel mice with their controls (WT and Padel) should reveal whether or not DA-neuron degeneration in MT and MT/Padel has occurred beforehand. Since there is no indication for such a loss of DA-neurons in one-year-old MT and MT/Padel mice in our study, so that an investigation of dying neurons does not seem to be rational.