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Mouse studies

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Abstract

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1 All mouse experiments were performed in accordance with the European Community Council Directive 2010/63/EU.

2 In compliance with Italian law (D. Lgs. n. 26/2014), the protocol was approved by the General Direction of Animal Health and Veterinary Drugs of the Italian Ministry of Health (authorization number 483/2023-PR).

3 C57BL/6n-Atm1Brd Pitrm1+/− mice were obtained from the Sanger Institute (http://www.informatics.jax.org/allele/MGI:5085349).

4 Animals were housed in groups of three per cage at 22 ± 2 °C, with a 12-hour light–dark cycle and 60% relative humidity.

5 Food (rodent standard diet; catalog number 4RF25, Mucedola, Settimo Milanese, Milan, Italy) and water were provided *ad libitum*.

6 The experimental design included two groups of female mice, aged 6 months and 15 months, consisting of 6 Pitrm1+/+ mice and 6 Pitrm1+/− mice.

7 For histological and immunohistochemical analyses, brains were fixed in formalin.

8 One hemisphere of each brain was sectioned and fresh-frozen for Western blot analysis.

9 For immunohistochemistry, the other hemisphere was frozen and embedded in optimal cutting temperature (OCT) compound (Tissue-Tek, Sakura Finetek, Torrance, CA, USA).

10 Brain slices of 20 µm thickness were obtained by cryosectioning.

11 Free-floating sections were stored in antifreeze solution.