様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

令和　５ 年　 ６ 月 １９ 日現在

機関番号：３２６２０
研究種目：若手研究
研究期間：2020～2022
課題番号：２０Ｋ１６４６８

研究課題名（和文）Functional analysis of astrocytes by using experimental autoimmune encephalomyelitis animal model (EAE)

研究課題名（英文）Functional analysis of astrocytes by using experimental autoimmune encephalomyelitis animal model (EAE)

研究代表者
ＣＯＳＳＵ　ＤＡＶＩＤＥ（Cossu, Davide）

順天堂大学・健康総合科学先端研究機構・准教授

研究者番号：９０８６７３２６

交付決定額（研究期間全体）：（直接経費）　　3,200,000円

研究成果の概要（和文）：我々は最近の研究で、E3ユビキチンリガーゼであるParkinが実験的自己免疫脳脊髄炎（EAE）時の末梢免疫細胞を介する免疫力を調節することを明らかにした。さらに、共通のミトコンドリア品質管理経路でParkinの上流に作用するPTEN誘導型推定キナーゼ1（PINK1）タンパク質が、EAE時の末梢炎症反応の調節に加齢に伴う役割を果たし、神経炎症および他の関連疾患の発症に寄与する可能性があることを明らかにしました。

さらに、BCG東京-172ワクチンが、積極的に誘導されたEAEモデルおよび自然発症のEAEモデルを抑制する有効性を実証した。

研究成果の学術的意義や社会的意義
We demonstrated that mutations in the genes related to mitochondria might contribute to the CNS inflammation, affecting peripheral and glia-dependent immune responses.
Our results also provided new insights into the neuroprotective mechanisms of BCG vaccine, useful for the development of therapies

研究成果の概要（英文）：Our recent research has shown that Parkin, an E3 ubiquitin ligase, modulates peripheral immune cells-mediated immunity during experimental autoimmune encephalomyelitis (EAE).
Furthermore, we demonstrated that PTEN-induced putative kinase 1 (PINK1) protein, which acts upstream of Parkin in a common mitochondrial quality control pathway, plays an age-related role in modulating the peripheral inflammatory response during EAE, potentially contributing to the pathogenesis of neuroinflammatory and other associated conditions.
Furthermore, we demonstrated the efficacy of BCG Tokyo-172 vaccine in suppressing actively induced and spontaneous EAE models. Age-related neurorotection was associated with reduced proliferation of splenic T-cells, an elevated frequency of interleukin-10 secreting CD8+ T cells in the spleen and a polarization of microglia and astrocytes towards an anti-inflammatory phenotype.

研究分野：neuroimmunology

キーワード：mitophagy　EAE　neuroinflammation　astrocytes　microglia　Parkin　Pink1　BCG

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

Neurodegeneration appears to be closely linked to neuroinflammation in several diseases, and a consistent factor across all these conditions is the prolonged activation of local microglia, astrocytes, and innate immune response. However, the specific pathophysiological mechanisms responsible for neurodegeneration remain largely unknown. Despite increasing evidence suggesting that reactive astrocytes have a dual phenotype, the precise molecular processes driving these changes are still subject to ongoing debate. Numerous clinical and preclinical studies have demonstrated alterations in the blood-brain barrier permeability among patients with neurodegenerative disorders, implying potential interactions between inflammatory cells in the peripheral system and astrocytes and microglia in the brain.

２．研究の目的

Given the numerous unresolved inquiries surrounding the intricate mechanisms through which inflammatory cells influence the initiation and advancement of neurodegeneration, as well as the role of astrocytes in the specific vulnerability of the central nervous system (CNS) to such diseases, the objective of this project is to shed light on the molecular and functional diversity of astrocytes and their interactions with microglia. Moreover, we studied the impact of mitophagy and aging on neuroinflammation and neurodegeneration. By doing so, we aim to unravel the underlying mechanisms responsible for the pathogenesis and progression of neurodegenerative disorders.

３．研究の方法

To accomplish our objective, we performed both clinical and animal studies. We employed the MOG-induced experimental autoimmune encephalomyelitis (EAE) model, that simulates acute inflammation within the central nervous system and is characterized by the disruption of the blood-brain barrier. We generated mice lacking the PARK2 and PINK1 genes, which are primarily responsible for facilitating the removal of damaged mitochondria through a process known as mitophagy. These mice were of varying age groups, including young, middle-aged, and elderly individuals. By utilizing techniques such as immunohistochemistry, T-cell proliferation assays, fluorescence-activated cell sorting, and enzyme-linked immunosorbent assay, we were able to gather substantial evidence elucidating the underlying mechanisms implicated in the development and progression of neurodegeneration. Importantly, our research shed new light on the functions of astrocytes in this context.

４．研究成果

Before we reported a statistically significant increase in the levels of PARKIN and PINK1 in the CSF and paired serum samples of patients with multiple sclerosis in the acute phase, highlighting the importance of mitophagy in the etiopathogenesis and progression of neuroinflammatory disorders (Figure 1).

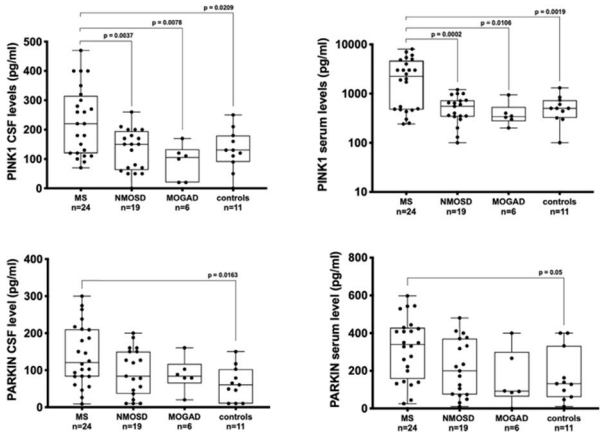

Figure. Concentration of PINK1 and PARKIN detected by ELISA in bodily fluids of patients with neuroinflammatory and neurodegenerative diseases and controlsubjects.

Concerning the animal research, the deletion of Parkin and PINK1 genes at a systemic level leads to an aggravation of EAE, characterized by increased disease severity. Mutations in these genes contribute to inflammation in the central nervous system, affecting both peripheral immune responses and immune responses mediated by glial cells. Notably, dendritic cells, neutrophils, and other innate immune cells may play a pivotal role in promoting the activation of microglia and astrocytes. The impairment of mitophagy, the process responsible for eliminating dysfunctional mitochondria, appears to modulate the functioning of the immune system within the CNS. Moreover, biological aging significantly influences the progression of autoimmune neuroinflammation. Remarkably, the age-related influence of Parkin and PINK1 proteins manifests in various subsets of innate and adaptive cells at different stages during active EAE development.

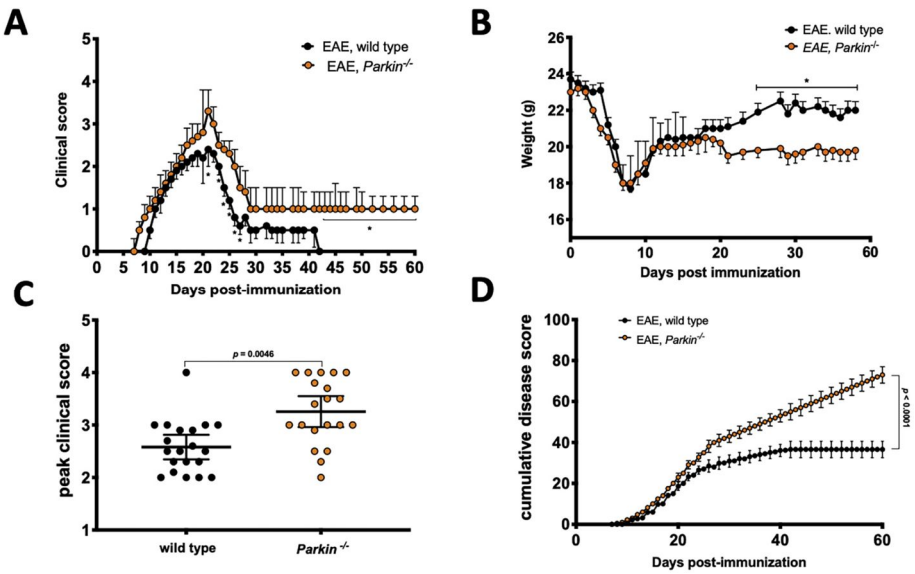

Figure. Clinical characteristics of EAE in wild type and Parkin knockout mice. Compared to the wild type controls, the Parkin knockout mice showed an earlier onset and greater severity of EAE with a greatly increased number of CD8αβ+TCRαβ+ T cells in the spleen and brain as well as a stronger T-cell proliferative response and an altered cytokine secretion in splenocytes.

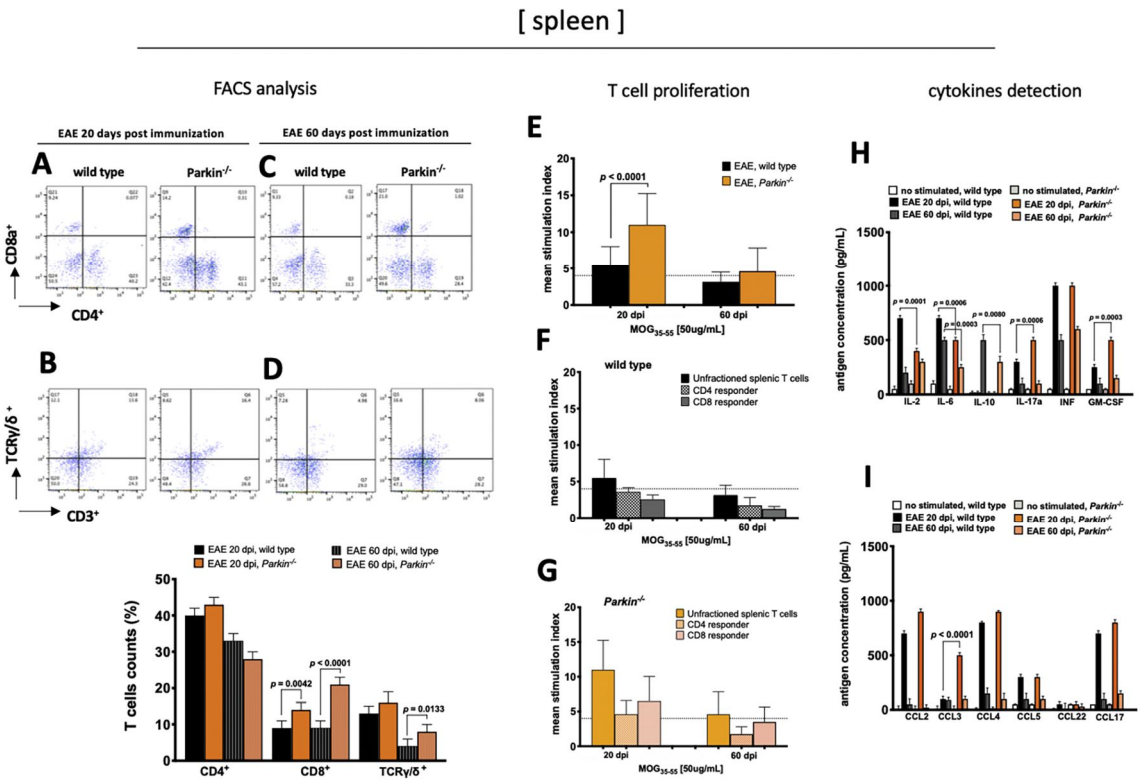

Figure. Distinct phenotypic profiles of T cells, cytokines, and T cell response in the spleen of Parkin knockout mice. Flow cytometry revealed an increased frequency of CD8+ (D, F) and γ/δ _T cells (E, G) in the spleen cells isolated from EAE mice during the acute and recovery phases of EAE.

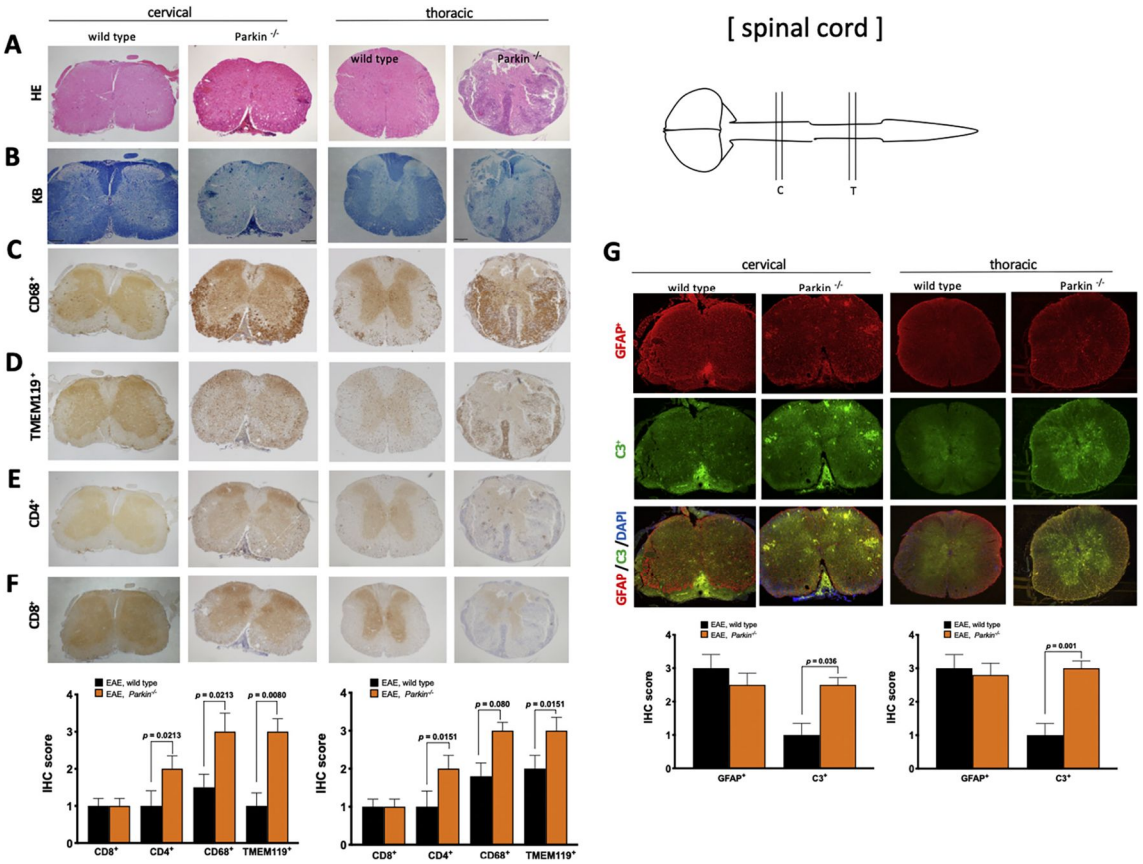

Figure. Histolology and immunohistochemistry of spinal cord of Parkin knockout mice, showitn infiltration of monocyte (C), strong microglia activation (D), and A1 astrocytes polarization (G)

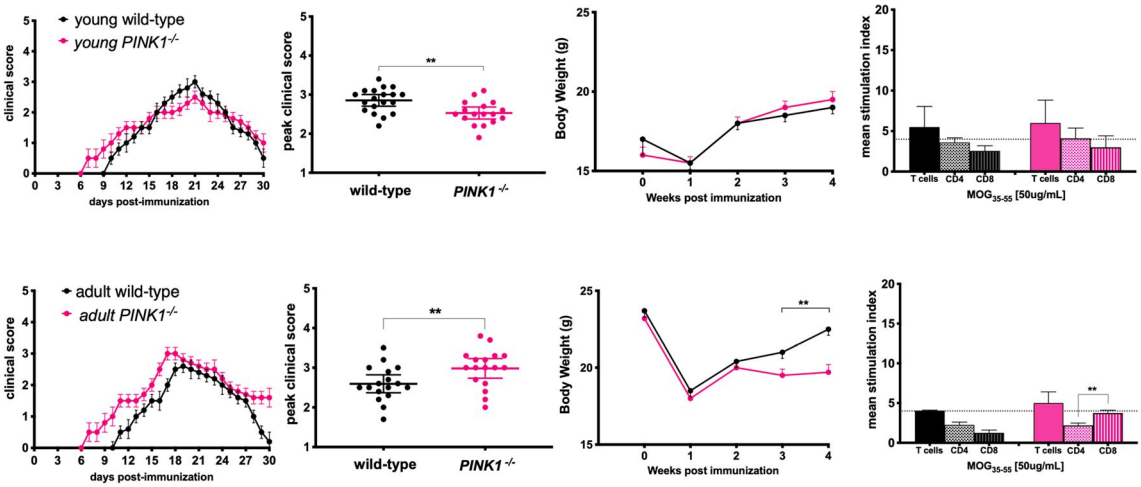

Figure. EAE development in PINK1knockout mice. Graph reports EAE mice clinical score evaluated daily. Compared to young wild-type controls, PINK1-/- mice showed earlier disease onset, albeit with a slightly less severe disease, while adult PINK1-/- mice displayed early onset and more severe acute symptoms than controls, showing persistent disease during the recovery phase. In adult mice, EAE severity was associated with significant increases in frequency of dendritic cells (CD11C+, IAIE+), lymphocytes (CD8+), neutrophils (Ly6G+, CD11b+),

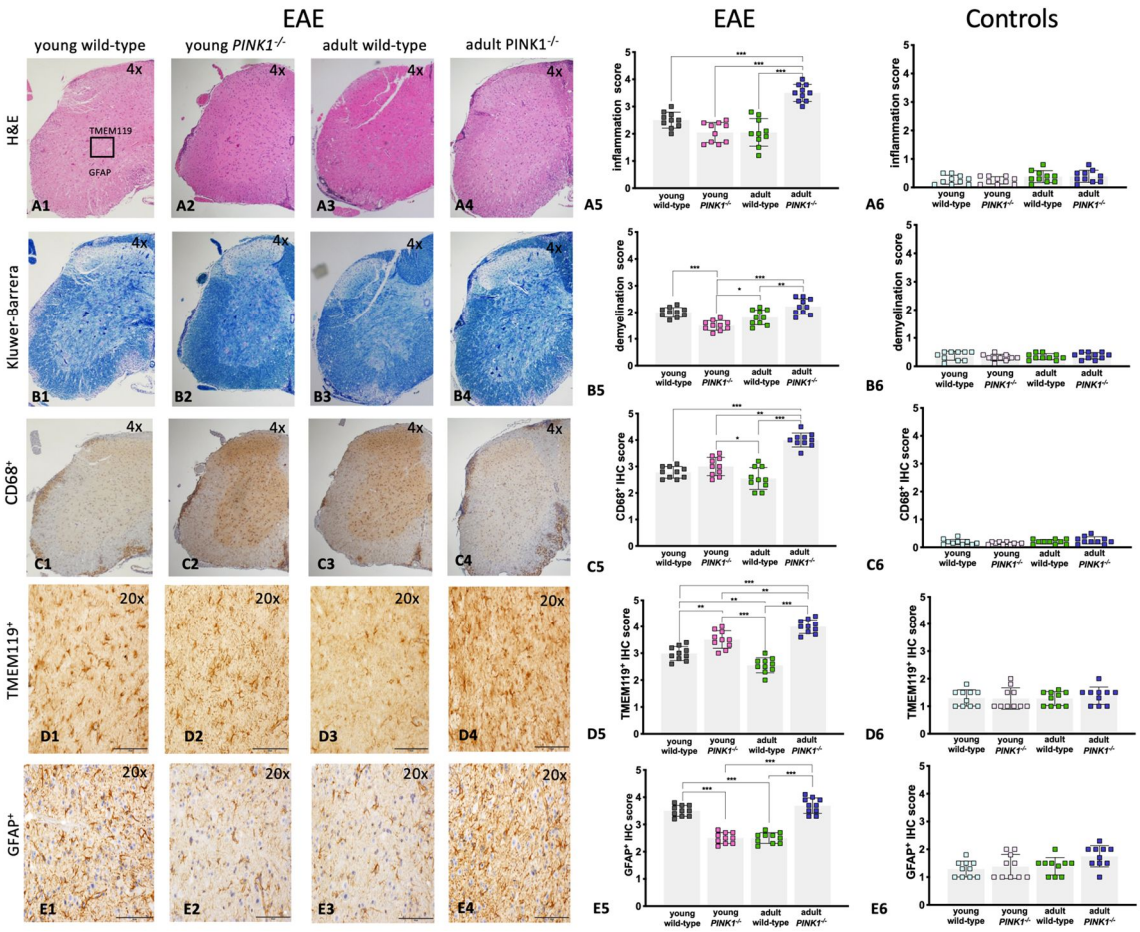

Figure. Histological and immunohistochemical analysis of the spinal cord tissue among groups during the EAE acute phase, showed a massive macrophage (CD68+) infiltration and microglia (TMEM119+) and astrocyte (GFAP+) in Pink1 knockout mice.

５．主な発表論文等

〔雑誌論文〕　計6件（うち査読付論文　0件／うち国際共著　0件／うちオープンアクセス　3件）

１．著者名 Cossu Davide、Yokoyama Kazumasa、Sakanishi Tamami、Sechi Leonardo A、Hattori Nobutaka	４．巻 0
２．論文標題 Bacillus Calmette?Gu?rin Tokyo-172 vaccine provides age-related neuroprotection in actively induced and spontaneous experimental autoimmune encephalomyelitis models	５．発行年 2023年
３．雑誌名 Clinical and Experimental Immunology	６．最初と最後の頁 uxad015
掲載論文のDOI（デジタルオブジェクト識別子） 10.1093/cei/uxad015	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Cossu Davide、Yokoyama Kazumasa、Sato Shigeto、Noda Sachiko、Sakanishi Tamami、Sechi Leonardo Antonio、Hattori Nobutaka	４．巻 13
２．論文標題 Age related immune modulation of experimental autoimmune encephalomyelitis in PINK1 knockout mice	５．発行年 2022年
３．雑誌名 Frontiers in Immunology	６．最初と最後の頁 1036680
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fimmu.2022.1036680	査読の有無 無
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

１．著者名 Cossu Davide、Ruberto Stefano、Yokoyama Kazumasa、Hattori Nobutaka、Sechi Leonardo A	４．巻 40
２．論文標題 Efficacy of BCG vaccine in animal models of neurological disorders	５．発行年 2022年
３．雑誌名 Vaccine	６．最初と最後の頁 432～436
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.vaccine.2021.12.005	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Cossu Davide、Watson Robert O.、Farina Cinthia	４．巻 12
２．論文標題 Editorial: A Microbial View of Central Nervous System Disorders: Interplay Between Microorganisms, Neuroinflammation and Behaviour	５．発行年 2021年
３．雑誌名 Frontiers in Immunology	６．最初と最後の頁 1～3
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fimmu.2021.816227	査読の有無 無
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

１．著者名 Cossu Davide、Yokoyama Kazumasa、Sato Shigeto、Noda Sachiko、Sechi Leonardo A.、Hattori Nobutaka	４．巻 359
２．論文標題 PARKIN modifies peripheral immune response and increases neuroinflammation in active experimental autoimmune encephalomyelitis (EAE)	５．発行年 2021年
３．雑誌名 Journal of Neuroimmunology	６．最初と最後の頁 577694～577694
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.jneuroim.2021.577694	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

１．著者名 Cossu Davide、Yokoyama Kazumasa、Sechi Leonardo Antonio、Hattori Nobutaka	４．巻 12
２．論文標題 Potential of PINK1 and PARKIN Proteins as Biomarkers for Active Multiple Sclerosis: A Japanese Cohort Study	５．発行年 2021年
３．雑誌名 Frontiers in Immunology	６．最初と最後の頁 1～7
掲載論文のDOI（デジタルオブジェクト識別子） 10.3389/fimmu.2021.681386	査読の有無 無
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 －

〔学会発表〕　計3件（うち招待講演　0件／うち国際学会　3件）

１．発表者名 Cossu Davide, Yokoyama Kazumasa, Hattori Nobutaka. Sechi Leonardo Antonio
２．発表標題 BCG-Tokyo 172 vaccination induces neuroprotection in spontaneous relapsing-remitting EAE
３．学会等名 41st Annual Congress of European Society of Mycobacteriology, (国際学会)
４．発表年 2021年

１．発表者名 Davide Cossu, Kazumasa Yokoyama, Shigeto Sato, Sachiko Noda, Nobutaka Hattori
２．発表標題 Lack of Parkin alters glial immune responses and increases neuroinflammation during EAE
３．学会等名 62th Annual Meeting of the Japanese Society of Neurology（国際学会）
４．発表年 2020年～2021年

１．発表者名
1.Cossu Davide, Yokoyama Kazumasa, Sato Shigeto, Noda Sachiko, Hattori Nobutaka

２．発表標題
Different peripheral immune modulation between Pink1-/- and Parkin-/-mice during EAE

３．学会等名
63回日本神経学会学術大会（国際学会）

４．発表年
2022年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

―

６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関