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Phosphorylated Rab12 reveals activity of the Parkinson's disease-related LRRK2 kinase in the Nucleus Basalis of Meynert

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Phosphorylated Rab12 reveals activity of the Parkinson's disease-related LRRK2 kinase in the Nucleus Basalis of Meynert

Abstract

Background: The Leucine Rich Repeat Kinase 2 (LRRK2) protein kinase is a large bifunctional enzyme with both GTPase and kinase activities. Mutations in the *LRRK2* gene are the most common familial genetic predisposition for Parkinson's disease (PD). LRRK2-PD patients present clinically similar to idiopathic PD (iPD) patients. All pathogenic mutations of LRRK2 increase their kinase activity (gain-of-function) against exogenous and endogenous substrates. Biochemical studies have identified a subset of Rab GTPases as bone fide substrates of LRRK2, that are hyperphosphorylated by LRRK2 PD mutants. The prevailing hypothesis is that LRRK2-PD mutant linked hyperphosphorylation of substrates underlies the onset of LRRK2 PD; due to clinical overlap, it is suspected that similar molecular pathogenicity may underly idiopathic PD. This presents two questions: 1) Where in human brain and what type of cells exhibit LRRK2 signaling? and 2) Is LRRK2 kinase-related signaling enhanced in LRRK2 PD and/or idiopathic PD.

To address in which brain region and cell type exhibit LRRK2 kinase signaling, we established immunocytochemical and immunohistochemical assays to detect substrate Rab phosphorylation in human autopsy brains. Though much work has elucidated the functional impact of Rab phosphorylation by LRRK2, we still lack spatial and cellular resolution of signaling events in the disease. In this study we examined the LRRK2→Rab12 signaling axis, in which Rab12 is phosphorylated by LRRK2 on Ser106.

Approach and Results: We validated a recombinant rabbit monoclonal antibody against Rab12 pSer106 (MJF-25-9) in immunocytochemistry for responsiveness to genetic and pharmacological modulators of LRRK2 kinase activity and found a strong correlation of pRab12 with LRRK2 kinase function. We next surveyed regions from throughout the human brain and found that although total Rab12 was detectable in multiple brain regions, pRab12 was only detected in the Nucleus Basalis of Meynert (NBM). Highly-multiplexed fluorescent imaging with the CODEX imaging technology was used to further correlate the localization of pRab12 in ChAT-positive cholinergic neurons of the NBM. Other regions, including substantia nigra which is a critical region in PD pathology, lack prominent pRab12 immunoreactivity across all of the experiments conducted here.

Conclusions: We established ICC, IHC and CODEX multiplex imaging techniques to observe LRRK2 signaling in autopsy brains and identified the NBM as a prominent site of LRRK2→Rab12 signaling. The NBM is a region implicated in cognitive dysfunction in Alzheimer's Disease and Parkinson's disease. Thus, our study offers a potential link between PD-associated LRRK2 mutation and cognition, through Rab12 phosphorylation.

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The Parkinson's disease Kinase LRRK2

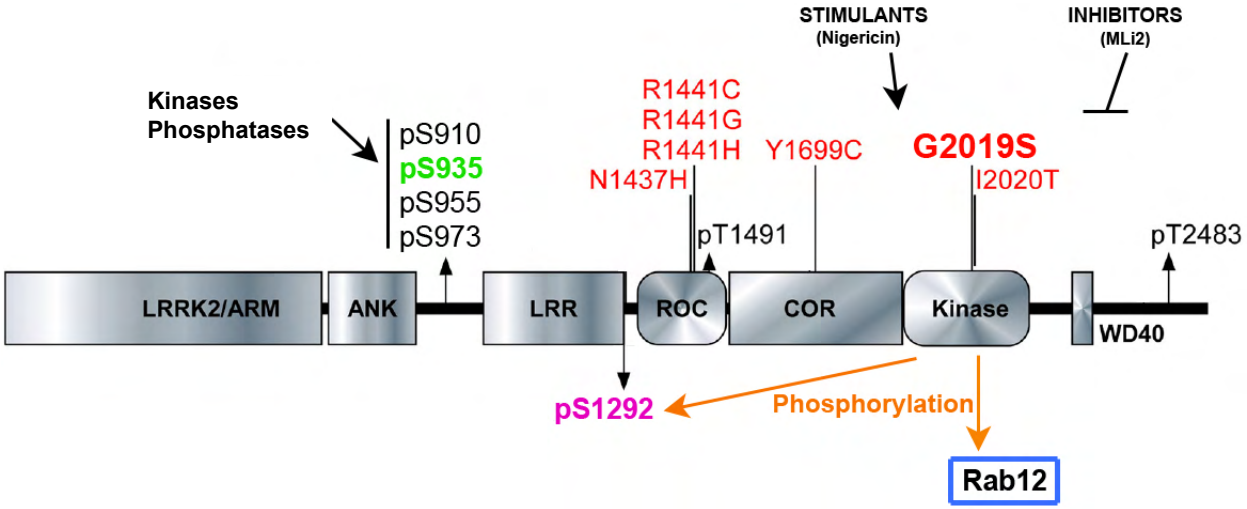

Figure 1. Representation of LRRK2 structure with its different domains (grey boxes) and PD-linked substitutions (red). The most common mutation in familial PD, G2019S, is shown in bold. Phosphorylation sites on LRRK2 are indicated in black. Autophosphorylation site at Ser1292 is shown in pink. Phosphorylation of Ser935 (green) is regulated by upstream kinase(s). LRRK2 phosphorylates Rab substrates, Rab10 and Rab12. In this study, we investigated pathogenic LRRK2-Rab12 signaling.

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Detection of pRab12 in LRRK2 over-expression conditions by immunoblot and PLA

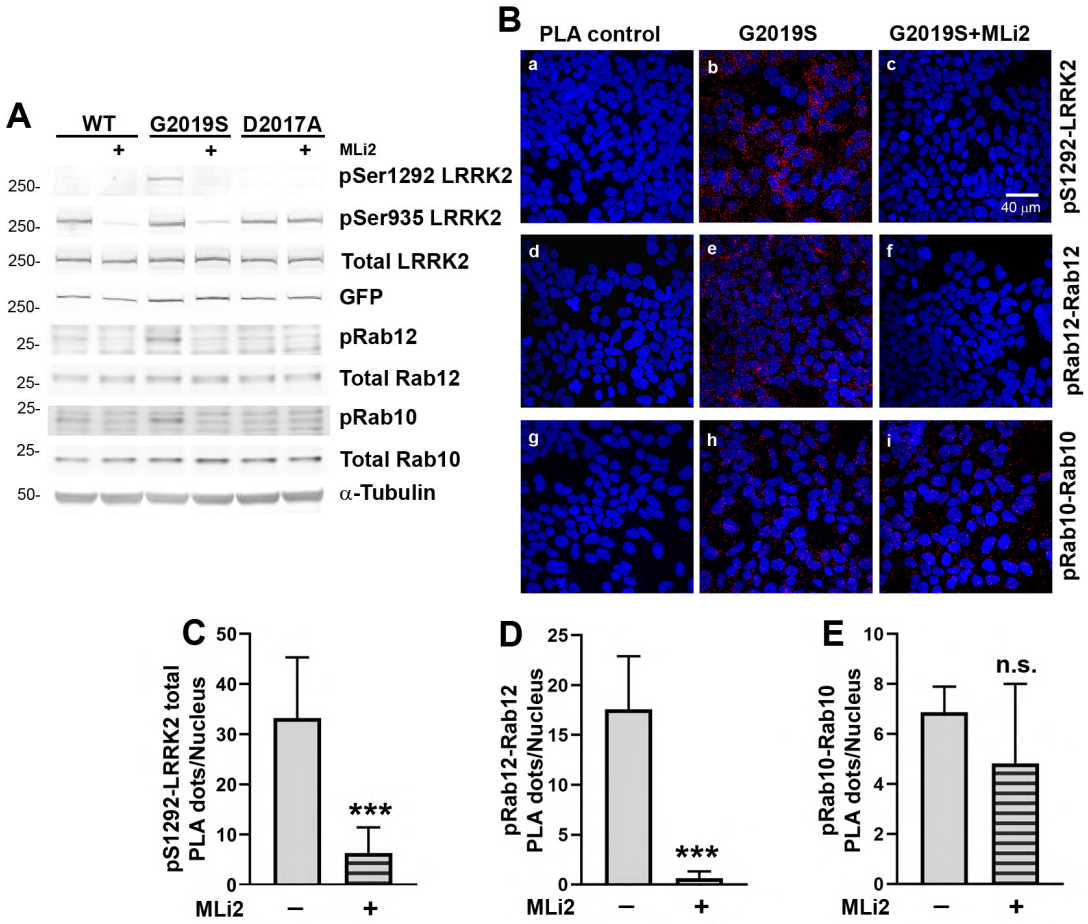

Figure 2. Effects of LRRK2 kinase inhibitor (MLi2) on LRRK2 and Rab substrates in TREx cells over-expressing shown LRRK2 variants. PLA (Proximity Ligation Assay) controls refer to samples that were either incubated with only one PLA probe **(a, g)** or Rab12 antibody alone **(d)**. Scale bar, 40 μm

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pRab12 is a reliable indicator of endogenous LRRK2 kinase activity by ICC

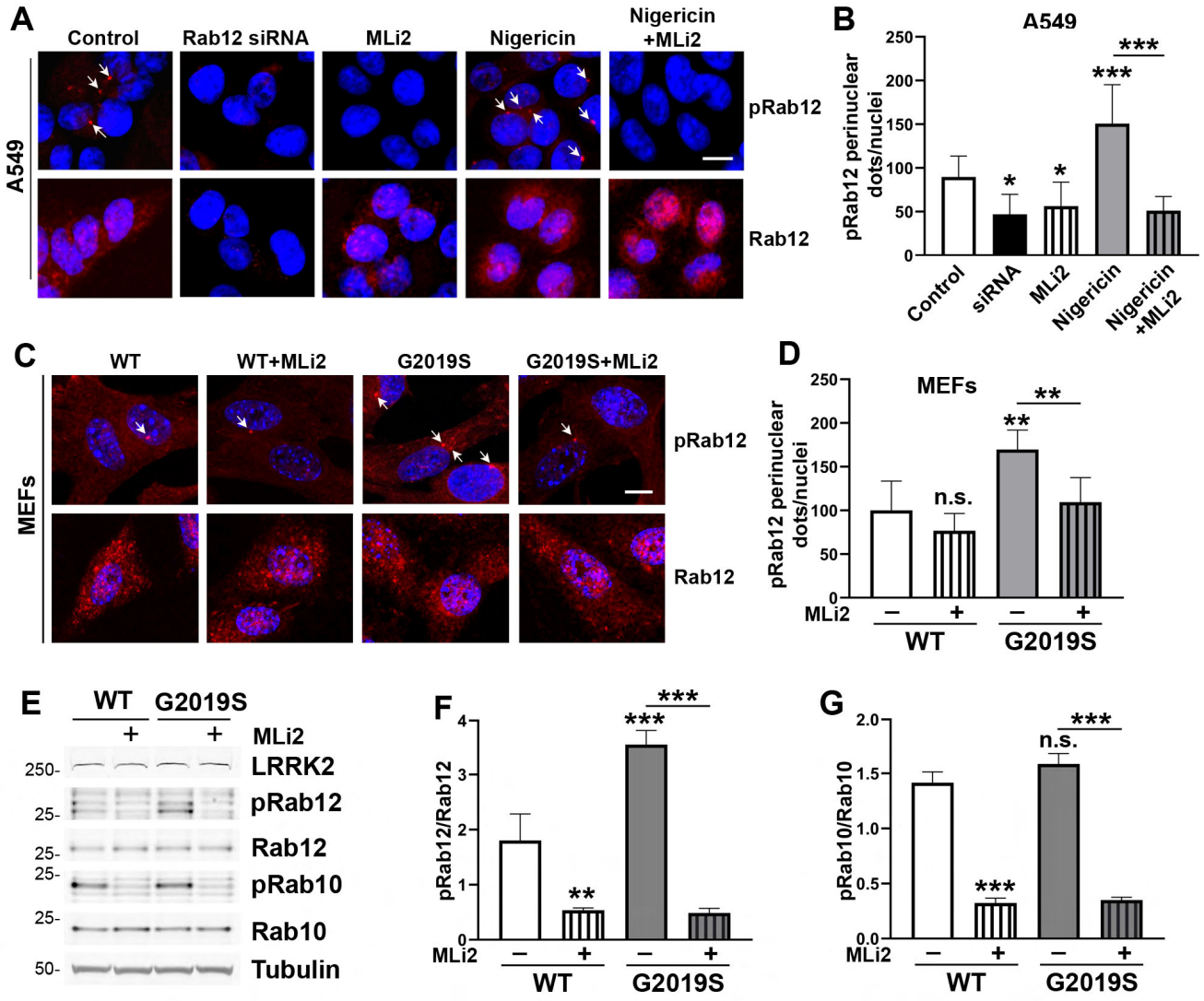

Figure 3. Impact of genetic and pharmacological modulation of LRRK2 kinase activity on A549 and MEF cells. **(A, B)** A549 cells exhibit endogenous LRRK2 kinase function. **(C-G)** MEF cells were harvested from transgenic mice expressing similar WT or G2019S LRRK2 levels. White arrows indicate pRab12-positive perinuclear punctae.

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Distribution of Rab12 and pRab12 in human brain

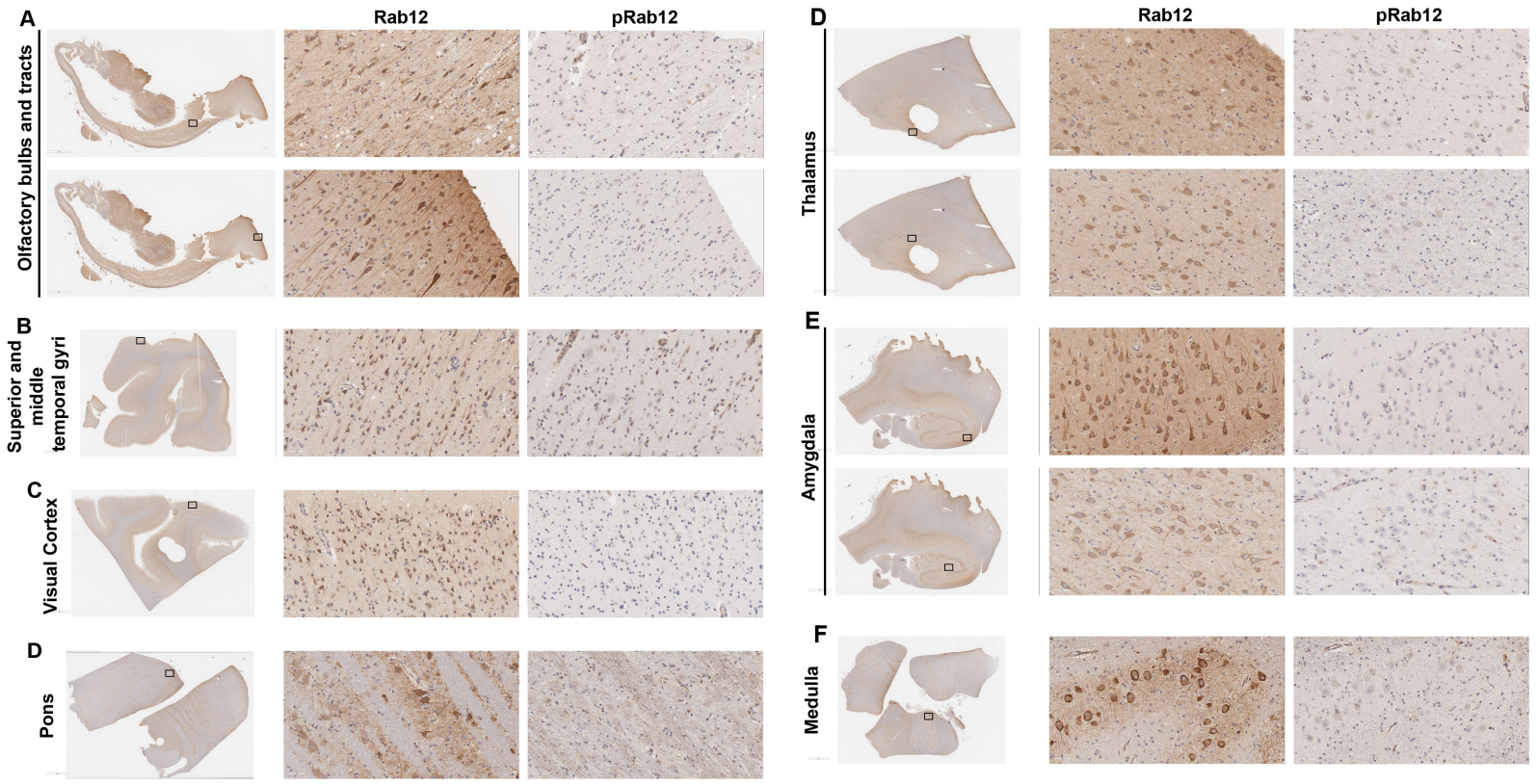

Figure 4. Representative IHC images of Rab12-positive regions and corresponding pRab12 staining in the same region of the designated brain sections. Scale bars, as shown.

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pRab12 localizes in the nucleus basalis of Meynert

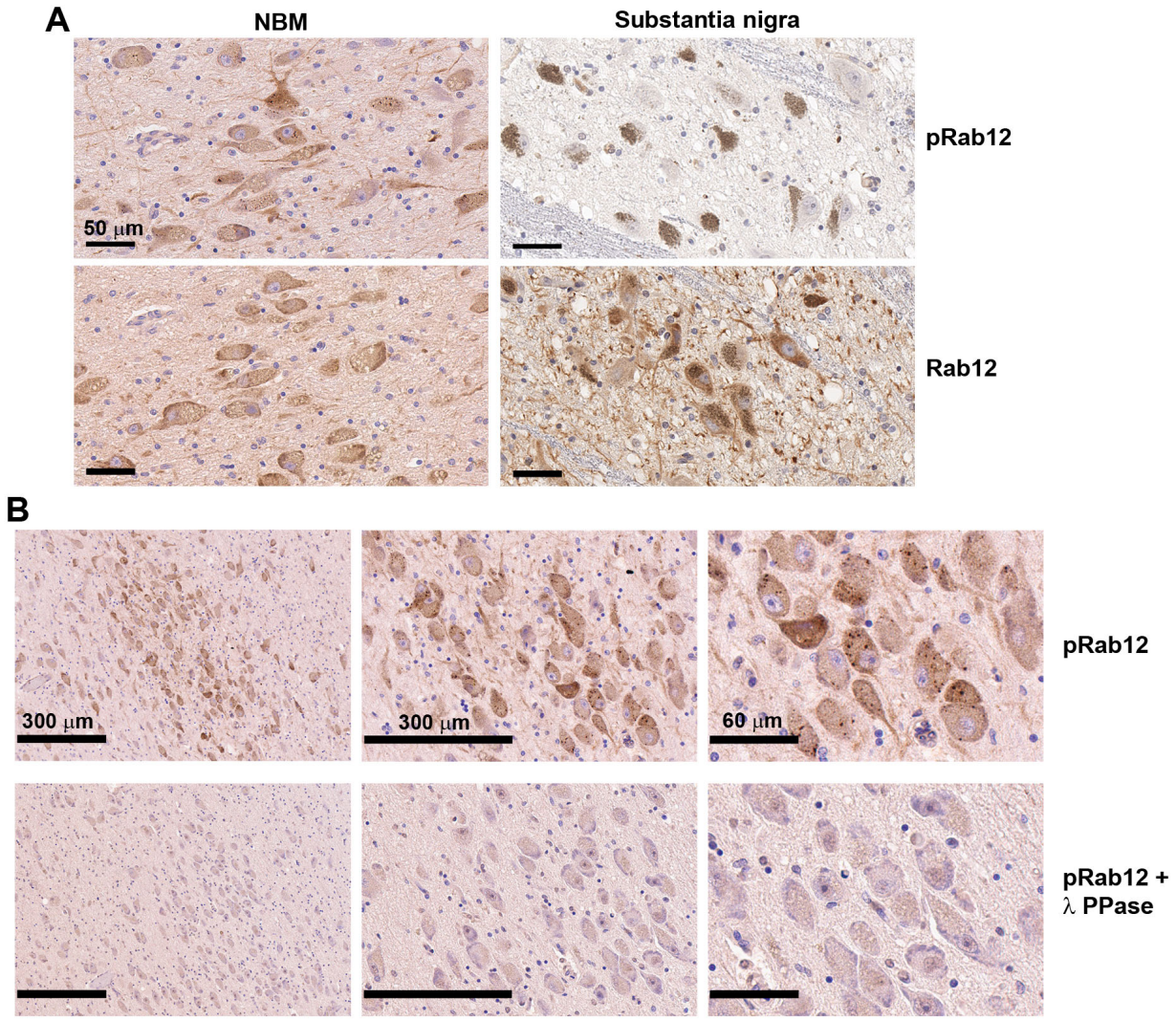

Figure 5. Representative pictographs of pRab12 and Rab12 staining in the NBM and substantia nigra. **(B)** Phospho-specificity of the pRab12 antibody assessed through treatment of brain sections with lambda phosphatase (λ PPase) in sections containing the NBM.

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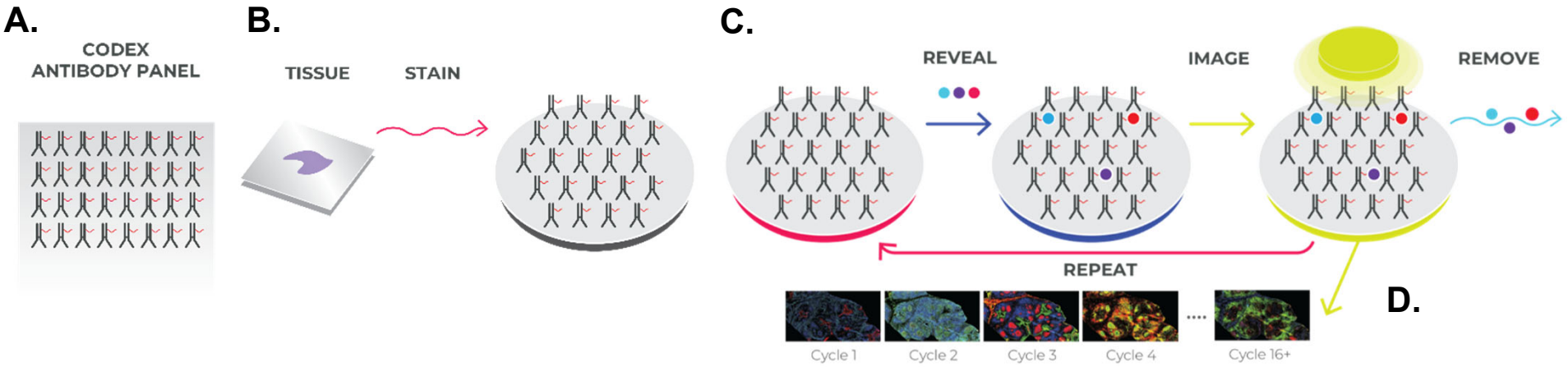
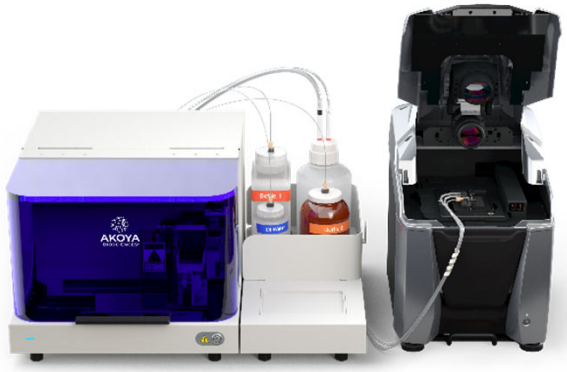
Identification of pRab12+ neuronal cell type using CODEX (co-detection by indexing)



E.



The CODEX® System

Figure 6. Codex workflow. A. Up to 40+ Antibodies are conjugated to DNA barcodes. **B.** Slides are stained with antibodies in a single-step. **C.** Antibody-antigen reactions are revealed by sequential detection of antibodies with fluorescent reporter and imaged. **D.** The cycle is repeated all within with the (**E.**) CODEX fluidics and imaging system to reveal all antigens.
In this study, we label and detect, ChAT, pRab12 and NeuN.

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pRab12 localizes in ChAT positive cholinergic neurons of the NBM

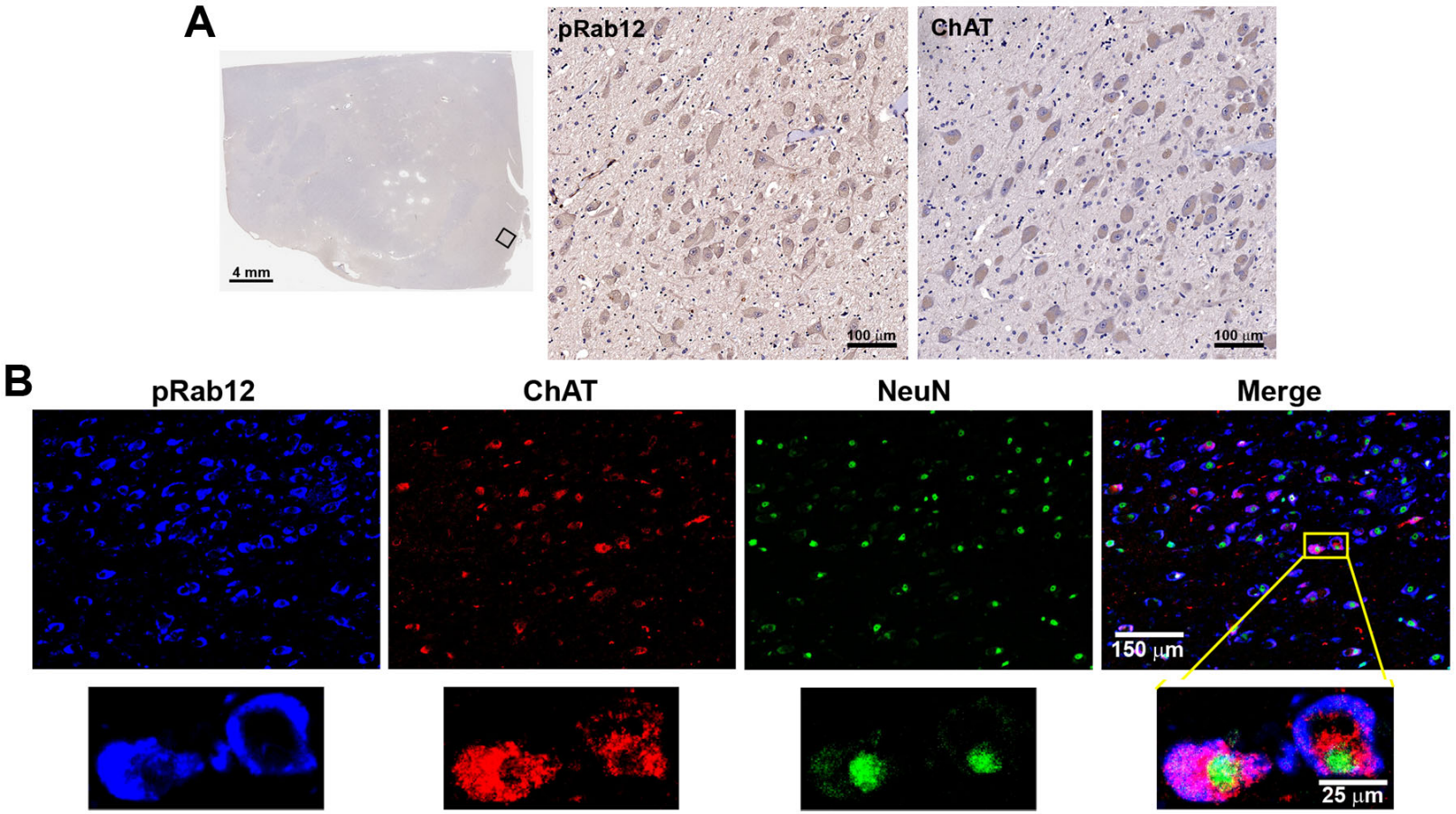

Figure 7. Staining of pRab12 and ChAT in **(A)** subsequent sections or **(B)** the same section of the NBM. **(B)** Multiplex fluorescent CODEX imaging was employed to evaluate localization of these two targets in the same neuron, along with NeuN general neuronal marker.

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Summary

- Rab12 phosphorylation is a reliable marker for LRRK2 kinase activity by immunoblot and ICC in model systems and in IHC in human brain.
- pRab12 is predominantly localized in the cholinergic neurons of the nucleus basalis of Meynert, a region implicated in cognitive function, early degeneration in Alzheimer's Disease and a prominent site for Lewy body detection
- pRab12 could provide a plausible link between LRRK2 mutation and reduced cognitive decline in PD

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