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# Polo-like kinase inhibition leads to neuroprotection of neurons bearing alpha-synuclein Lewy body-like inclusions *in vivo*

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## Abstract

α-synuclein (αSyn) and S129 phosphorylated αSyn (pSyn) define synucleinopathies like Parkinson's disease (PD). Targeting S129 αSyn kinases, like the Polo-like kinase (PLK) family, could provide a therapeutic strategy to limit degeneration of cells bearing aggregated αSyn inclusions. Using longitudinal *in vivo* multiphoton imaging in mouse cortex after αSyn inclusion induction, we find an increase in cell survival of inclusion-bearing neurons after PLK inhibition. PLK inhibition is associated with increased αSyn levels within inclusions and increased nuclear DNA damage repair markers. Overall, these findings suggest that PLK inhibition may serve as a potential therapeutic strategy for limiting neurodegeneration in PD.

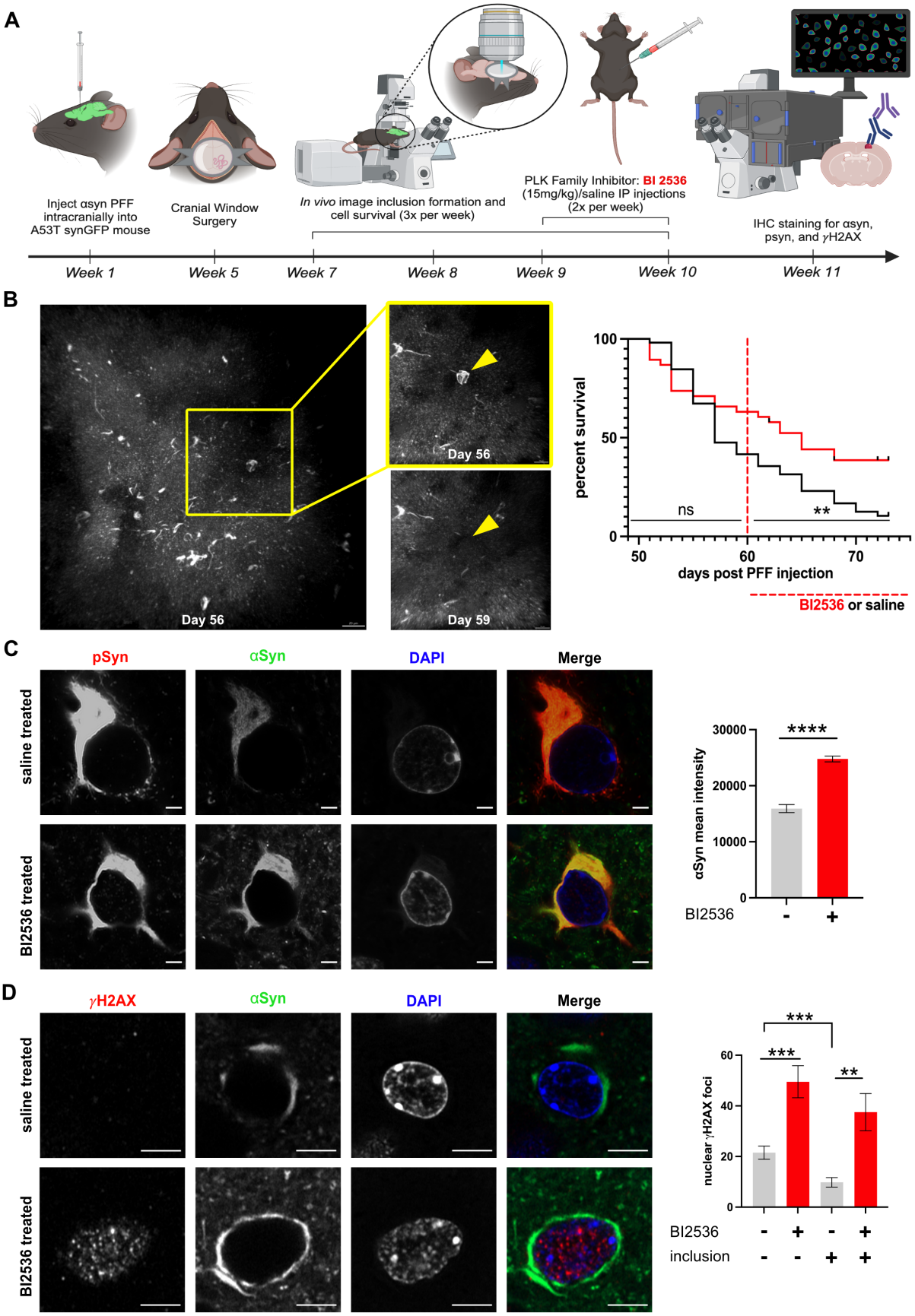


**Figure 1. PLK inhibition protects against neurodegeneration in neurons bearing somatic Lewy inclusions *in vivo*:**

A) Cartoon schematic of experimental protocol to induce Lewy pathology in mouse cortex via intracranial injection of αSyn pre-formed fibrils (PFFs). *In vivo* imaging was performed after cranial window surgery for 4 weeks, imaging each cortical brain region 3x per week. Baseline imaging consisted of 2 initial weeks and then mice were either injected with PLK inhibitor BI2536 or saline 2x per week for 2 weeks. IHC was then performed on dissected brains after drug treatment. Created with BioRender.com. B) Representative images of mouse brain cortex *in vivo* demonstrating loss of cell body bearing αSyn somatic inclusion (yellow arrowhead) from day 55 to day 59 post PFF injection. Scale bar 20mm (LEFT), scale bar 10mm (MIDDLE). Images were taken as separate acquisitions of inclusions (top 21mm z-stack, bottom 36mm z-stack). RIGHT: Survival curve of somatic inclusions across 25 days of longitudinal imaging of cortical regions *in vivo* in mice treated with saline or PLK 1/2/3 Inhibitor BI2536 (15mg/kg) IP injections for 2 weeks starting day 60 post PFF injection. Overall Mantel-cox test p=0.0161. Pre BI2536/saline treatment p=0.1454. Post BI2536/saline treatment p<0.0055. Saline treated group N= 4 animals. BI2536 treated group N=4 animals. 108 inclusions counted. C) BI2536 treatment (15mg/kg IP injections twice per week for two weeks) is associated with no change in pSyn levels, but increased total αSyn levels within PFF-induced aggregated somatic inclusion. Scale bar 2mm. RIGHT: Quantification of synuclein levels within the aggregate. Significant increase of αSyn mean intensity from BI2536 treated mice (24755 ±511.682) compared to saline treated mice (15920 ±707.861)(p<0.0001). No significant difference between pSyn mean intensity from saline treated mice (21766 ±1255.436) and BI2536 treated mice (19698 ±918.248)(p=0.1805), data upon request. No significant difference of volume of the inclusion between saline treated mice (126.6 ±10.803) and BI2536 treated mice (133.6 ±6.606)(p=0.5559), data upon request. N= 5-6 mice in each group, n=143 inclusions. Two-tailed student's t-test. D) BI2536 treatment is associated with increased DSB levels in PFF-induced cortical Lewy pathology mouse model. Scale bar 5mm. RIGHT: Quantification. Significant increase of nuclear γH2AX foci in cells without inclusions from BI2536 treated mice (49.55 ±6.334) compared to cells without inclusions from saline treated mice (21.54 ±2.605)(p=0.0007), but no significant difference when compared to cells bearing inclusions from BI2536 treated mice (37.53 ±7.357)(p=0.2178). Significant increase of nuclear γH2AX foci in BI2536 treated cells bearing inclusions compared to saline treated cells bearing inclusions (9.812 ±1.886)(p=0.0031). Significant decrease of nuclear γH2AX foci in saline treated cells bearing inclusions compared to cells without inclusions (p=0.0004). N= 5-6 mice in each group, n=349 inclusions. Two-tailed student's t-test.

## Description

A hallmark of Parkinson's disease (PD) and other synucleinopathies is α-synuclein (αSyn) aggregation into cytoplasmic inclusions, called Lewy bodies, found within surviving neurons in brain regions susceptible to cell death. It remains unknown, however, how Lewy body pathology, which is enriched in S129 phosphorylated αSyn (pSyn), contributes to cell death. pSyn is the most common post-translational modification αSyn undergoes and is highly enriched in Lewy pathology (Anderson et al., 2006), but it is unclear whether pSyn promotes or protects against aggregation and/or cell death (Tenreiro et al., 2014). While S129 phosphorylation has been shown to reduce αSyn fibrillogenesis *in vitro* (Paleologou et al., 2008), other *in vitro* studies suggest that it can also promote fibrillar aggregation depending on the exact conditions used (Fujiwara et al., 2002; Ma et al., 2016; Samuel et al., 2016). *In vivo*, studies utilizing a phospho-deficiency approach where S129 is mutated to alanine increased aggregation in drosophila (L. Chen & Feany, 2005), but in rat brain had no effect (McFarland et al., 2009) or reduced (Gorbatyuk et al., 2008) aggregation compared to the phospho-mimic S129D mutation. Another strategy to study the effects of S129 phosphorylation is to modulate the kinases and phosphatases that act at this residue. Several kinase families have been shown to produce pSyn *in vitro* (Ishii et al., 2007; Kawahata et al., 2022; Pronin et al., 2000; Qing et al., 2009; Wu et al., 2020), but several studies, including our own work, suggest that the Polo-Like Kinase (PLK) family members 1, 2, and 3 (Aubele et al., 2013; Basso et al., 2013; Bergeron et al., 2014; Inglis et al., 2009; Mbefo et al., 2010; Oueslati et al., 2013; Waxman & Giasson, 2011) may be the most important *in vivo* (Weston et al., 2021). Our previous work demonstrated that genetic knockout of PLK2 led to improved survival of cortical neurons bearing aggregated αSyn Lewy body-like inclusions in mouse cortex *in vivo* (Weston et al., 2021). Given the attractiveness of kinase inhibitors as potential therapeutic agents, we set out here to test the effect of PLK inhibition on αSyn biology and neurodegeneration.

In order to test how manipulating phosphorylation of αSyn may affect cell death, we measured cell survival of Lewy inclusion-containing neurons longitudinally over 4 weeks, utilizing an *in vivo* multiphoton imaging approach in the A53T Syn-GFP mouse line (Schaser et al., 2020)(Fig. 1A). Previous work shows that the GFP-tagging does not detectibly affect synuclein aggregation in this experimental paradigm (Osterberg et al., 2015; Spinelli et al., 2014). Mouse cortical regions were imaged for a 2-week baseline period, and then for an additional 2 weeks during exposure to the pan-PLK1-3 inhibitor BI2536 or saline control. PLK inhibition started at day 60 after intracortical αSyn preformed fibril (PFF) injection to induce Lewy pathology, a time point we have previously shown leads to robust cortical pathology that can be imaged *in vivo* (Schaser et al., 2020). No significant differences in the rate of Lewy inclusion-bearing neuron cell death were detected between the two groups of mice during the baseline imaging period before PLK inhibition. However, during the 2-week BI2536 treatment

period, we measured an increase in survival rate of Lewy inclusion-bearing cells treated with BI2536 compared to saline control (Fig. 1B). These results suggest that acute pharmacologic inhibition of PLK protects against neurodegeneration of neurons bearing Lewy inclusions and extends our previous result in PLK2 KO mice (Weston et al., 2021), now with a clinically relevant treatment paradigm.

To investigate the effects of PLK inhibition on aggregated αSyn within Lewy pathology, we used fixed tissue immunohistochemstry (IHC) to study neuronal somatic inclusions from mouse cortex after BI2536 or saline treatment after our *in vivo* imaging experiment (Fig. 1B) had ended. Interestingly, BI2536 treatment increased the level of total αSyn protein within Lewy inclusions, but had no significant effect on pSyn levels (data reported in figure legend) (Fig. 1C). This suggests that PLK1, 2 and 3 are not S129 αSyn kinases for Lewy pathology, but do alter αSyn protein levels within inclusions. Previous work has suggested that PLK inhibition decreases αSyn degradation within aggregates (Oueslati et al., 2013). Our data are consistent with this result. Because of the growing body of literature linking αSyn to DNA double-strand break (DSB) repair (Arnold et al., 2024; H.-Y. Chen et al., 2024; Rose et al., 2024; Schaser et al., 2019) and previous work showing that PLK2 KO led to increased αSyn in nuclear double-strand break (DSB) repair foci (Weston et al., 2021), we next tested whether PLK inhibition changes levels of the DSB repair marker, gH2AX. Via IHC, we found that BI2536 treatment caused an increase in gH2AX levels, both in cells with and without somatic Lewy inclusions (Fig. 1D). How PLK inhibition leads to an increase in gH2AX is not clear; it could be due to a specific effect on mediators of DSB repair like C-terminal binding protein interacting protein (CtIP), which are known to be phosphorylated by PLK (Barton et al., 2014; Wang et al., 2018) or potentially through its effect to decrease αSyn degradation within cells.

In summary, our *in vivo* multiphoton imaging data show that inhibiting PLK acutely can improve survival of Lewy inclusion-bearing neurons in cortex. This extends our previous work in PLK2 KO mice (Weston et al., 2021) by suggesting that pharmacologic inhibition may have similar effects and be potentially therapeutic. Our previous work in PLK2 KO mice demonstrated that PLK2 was not a Lewy pathology kinase (Weston et al., 2021) and our new data with BI2536, which inhibits PLK1, 2, & 3 further suggests that neither PLK1 or 3 is a Lewy pathology kinase either. We did detect an increase in total aggregated αSyn levels within inclusions after PLK inhibition, however, which is consistent with previous work from Lashuel and colleagues that finds that PLK2 regulates and enhances autophagic clearance of αSyn in a kinase-dependent manner (Oueslati et al., 2013). More investigation is required to decipher how PLK inhibition leads to increased neuronal survival and whether DSB repair and genomic stability play a role, but it is interesting to speculate that increases in αSyn levels within the inclusion may be mirrored by increases in soluble nuclear αSyn as well that could be promoting more efficient DSB repair.

Together, our data shows that acute PLK inhibition can lead to neuroprotection in a Lewy pathology model and understanding this mechanism better could lead to new treatments for clinically important forms of neurodegenerative disease.

## Methods

### Animals.

All mice lines were housed in OHSU's Department of Comparative Medicine (DCM) facilities in a light-dark cycle vivarium. Animals were maintained under *ad libitum* food and water diet. All animal protocols were approved by OHSU IACUC, and all experiments were performed with every effort to reduce the number of animal used and their suffering. The A53T-Syn-GFP mouse line was genetically created (Schaser et al., 2019) and characterized (Schaser et al., 2020) according to our previous research.

### Mouse brain *in vivo* imaging & analysis.

2 to 3 month-old male and female mice were unilaterally injected with 2.5mL (2mg/mL) of freshly sonicated mouse WT sequence PFFs using the same protocol as we have previously published (Schaser et al., 2019). Cranial window surgeries were performed 5 weeks post PFF injection according to our previous published protocols. This timeline was chosen based on previous research with this A53T transgenic mouse that has accelerated pathology spread for optimal imaging (Schaser et al., 2020). Mouse cortex was imaged 3 weeks post cranial window surgery using a Zeiss LSM 7MP multiphoton microscope. Zeiss Zen image acquisition software was used to collect z-stacks from layer 1 to layer 2/3 of the cortex with 3µm intervals at 63x zoom 1. Regions of interest (ROIs) were analyzed in FIJI and inclusions were verified visually for each day of imaging by hand. New inclusions were counted for each day of imaging and scored by hand. No detectible sex differences were observed. Survival curves were created with Prism10 (GraphPad). Cortical regions were imaged for 4 weeks at 3 times per week. After 2 weeks animals were given a 2 week treatment of saline or 15mg/kg BI2536 IP injections twice per week. Animals were sacrificed after 4 weeks of imaging for IHC.

### Immunohistochemistry.

Mouse Tissue: Brains from 4-5 month old mice were dissected and fixed according to previously published protocols (Schaser et al., 2019). Brains were sectioned into 50mM coronal slices using a Vibratome LeicaVT1000S. Tissue was fixed, permeabilized, incubated in blocking buffer, stained with primary and secondary antibodies, and mounted as previously published (Schaser et al., 2019). Imaging was performed on a Zeiss 980 laser-scanning confocal microscope with a 63x oil objective zoom 7.0. Z-stacks of the αSyn inclusion and nucleus with optimal intervals. Analysis was performed in IMARIS using a 3D surface reconstruction of the inclusion and the DAPI channel to create a nuclear mask. We then used IMARIS to quantify the nuclear $\gamma$H2AX foci count or αSyn and pSyn levels with an average intensity measurement.

Primary antibodies used were: anti-Syn1, 1:500 dilution, mouse monoclonal, BD Biosciences, cat. 610786; anti-Phospho-Histone H2a.X, 1:500 dilution, rabbit monoclonal, Cell Signaling, cat. 9718; anti-PhosphoS129-Syn EPY1536Y, 1:500 dilution, rabbit monoclonal, Abcam ab51253. Secondary antibodies used were: Alexa Fluor 555 goat anti-mouse, Abcam ab150114; Alexa Fluor 647 donkey anti-rabbit, Jackson ImmunoResearch Laboratories 711605152.

## Reagents

| **Animal** | **Strain Genetic Background** | **Gene with Mutation** | **Genotype** | **Obtained** |
|---|---|---|---|---|
| A53T-Syn-GFP mouse | A53TGFPXC57/B6 | SNCA (αSyn) | A53T $^{+}$/A53T $^{+}$ | Created in house |

| **Primary Antibody** | **Description** | **Source** |
|---|---|---|
| Syn1 | Mouse monoclonal, 1:500 dilution, specific to αSyn | BD Biosciences, 610786 |
| $\gamma$H2AX | Rabbit monoclonal, 1:500 dilution, specific to phosphor-Histone H2a.X | Cell Signaling, 9718 |
| pSyn | Rabbit monoclonal, 1:500 dilution, specific to PhosphoS129-Syn EPY1536Y | Abcam, ab51253 |

| **Secondary Antibody** | **Description** | **Source** |
|---|---|---|
| Mouse IgG | Goat polyclonal, 1:1000 dilution | Abcam ab150114 |
| Rabbit IgG | Donkey polyclonal, 1:1000 dilution | Jackson ImmunoResearch Laboratories 711605152 |

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## References

Anderson JP, Walker DE, Goldstein JM, de Laat R, Banducci K, Caccavello RJ, et al., Chilcote. 2006. Phosphorylation of Ser-129 Is the Dominant Pathological Modification of α-Synuclein in Familial and Sporadic Lewy Body Disease. Journal of

Biological Chemistry 281: 29739-29752. DOI: 10.1074/jbc.M600933200

Arnold MR, Cohn GM, Oxe KC, Elliott SN, Moore C, Laraia PV, et al., Unni. 2024. Alpha-synuclein regulates nucleolar DNA double-strand break repair in melanoma. : 10.1101/2024.01.13.575526. DOI: 10.1101/2024.01.13.575526

Aubele DL, Hom RK, Adler M, Galemmo RA, Bowers S, Truong AP, et al., Artis. 2013. Selective and Brain-Permeable Polo-like Kinase-2 (Plk-2) Inhibitors That Reduce α-Synuclein Phosphorylation in Rat Brain. ChemMedChem 8: 1295-1313. DOI: 10.1002/cmdc.201300166

Barton O, Naumann SC, Diemer-Biehs R, Künzel J, Steinlage M, Conrad S, et al., Löbrich. 2014. Polo-like kinase 3 regulates CtIP during DNA double-strand break repair in G1. Journal of Cell Biology 206: 877-894. DOI: 10.1083/jcb.201401146

Basso E, Antas P, Marijanovic Z, Gonçalves S, Tenreiro S, Outeiro TF. 2013. PLK2 Modulates α-Synuclein Aggregation in Yeast and Mammalian Cells. Molecular Neurobiology 48: 854-862. DOI: 10.1007/s12035-013-8473-z

Bergeron M, Motter R, Tanaka P, Fauss D, Babcock M, Chiou Ss, et al., Anderson. 2014. In vivo modulation of polo-like kinases supports a key role for PLK2 in Ser129 α-synuclein phosphorylation in mouse brain. Neuroscience 256: 72-82. DOI: 10.1016/j.neuroscience.2013.09.061

Chen HY, Liao CY, Li H, Ke YC, Lin CH, Teng SC. 2024. ATM-mediated co-chaperone DNAJB11 phosphorylation facilitates α-synuclein folding upon DNA double-stranded breaks. NAR Molecular Medicine 1: 10.1093/narmme/ugae007. DOI: 10.1093/narmme/ugae007

Chen L, Feany MB. 2005. α-Synuclein phosphorylation controls neurotoxicity and inclusion formation in a Drosophila model of Parkinson disease. Nature Neuroscience 8: 657-663. DOI: 10.1038/nn1443

Fujiwara H, Hasegawa M, Dohmae N, Kawashima A, Masliah E, Goldberg MS, et al., Iwatsubo. 2002. α-Synuclein is phosphorylated in synucleinopathy lesions. Nature Cell Biology 4: 160-164. DOI: 10.1038/ncb748

Gorbatyuk OS, Li S, Sullivan LF, Chen W, Kondrikova G, Manfredsson FP, Mandel RJ, Muzyczka N. 2008. The phosphorylation state of Ser-129 in human α-synuclein determines neurodegeneration in a rat model of Parkinson disease. Proceedings of the National Academy of Sciences 105: 763-768. DOI: 10.1073/pnas.0711053105

Inglis KJ, Chereau D, Brigham EF, Chiou SS, Schöbel S, Frigon NL, et al., Anderson. 2009. Polo-like Kinase 2 (PLK2) Phosphorylates α-Synuclein at Serine 129 in Central Nervous System. Journal of Biological Chemistry 284: 2598-2602. DOI: 10.1074/jbc.C800206200

Ishii A, Nonaka T, Taniguchi S, Saito T, Arai T, Mann D, et al., Hasegawa. 2007. Casein kinase 2 is the major enzyme in brain that phosphorylates Ser129 of human α-synuclein: Implication for α-synucleinopathies. FEBS Letters 581: 4711-4717. DOI: 10.1016/j.febslet.2007.08.067

Kawahata I, Finkelstein DI, Fukunaga K. 2022. Pathogenic Impact of α-Synuclein Phosphorylation and Its Kinases in α-Synucleinopathies. International Journal of Molecular Sciences 23: 6216. DOI: 10.3390/ijms23116216

Ma MR, Hu ZW, Zhao YF, Chen YX, Li YM. 2016. Phosphorylation induces distinct alpha-synuclein strain formation. Scientific Reports 6: 10.1038/srep37130. DOI: 10.1038/srep37130

Mbefo MK, Paleologou KE, Boucharaba A, Oueslati A, Schell H, Fournier M, et al., Lashuel. 2010. Phosphorylation of Synucleins by Members of the Polo-like Kinase Family. Journal of Biological Chemistry 285: 2807-2822. DOI: 10.1074/jbc.M109.081950

McFarland NR, Fan Z, Xu K, Schwarzschild MA, Feany MB, Hyman BT, McLean PJ. 2009. α-Synuclein S129 Phosphorylation Mutants Do Not Alter Nigrostriatal Toxicity in a Rat Model of Parkinson Disease. Journal of Neuropathology & Experimental Neurology 68: 515-524. DOI: 10.1097/NEN.0b013e3181a24b53

Osterberg VR, Spinelli KJ, Weston LJ, Luk KC, Woltjer RL, Unni VK. 2015. Progressive Aggregation of Alpha-Synuclein and Selective Degeneration of Lewy Inclusion-Bearing Neurons in a Mouse Model of Parkinsonism. Cell Reports 10: 1252-1260. DOI: 10.1016/j.celrep.2015.01.060

Oueslati A, Schneider BL, Aebischer P, Lashuel HA. 2013. Polo-like kinase 2 regulates selective autophagic α-synuclein clearance and suppresses its toxicity in vivo. Proceedings of the National Academy of Sciences 110: 10.1073/pnas.1309991110. DOI: 10.1073/pnas.1309991110

Paleologou KE, Schmid AW, Rospigliosi CC, Kim HY, Lamberto GR, Fredenburg RA, et al., Lashuel. 2008. Phosphorylation at Ser-129 but Not the Phosphomimics S129E/D Inhibits the Fibrillation of α-Synuclein. Journal of Biological Chemistry 283: 16895-16905. DOI: 10.1074/jbc.M800747200

Pronin AN, Morris AJ, Surguchov A, Benovic JL. 2000. Synucleins Are a Novel Class of Substrates for G Protein-coupled Receptor Kinases. Journal of Biological Chemistry 275: 26515-26522. DOI: 10.1074/jbc.M003542200

Qing H, Wong W, McGeer EG, McGeer PL. 2009. Lrrk2 phosphorylates alpha synuclein at serine 129: Parkinson disease implications. Biochemical and Biophysical Research Communications 387: 149-152. DOI: 10.1016/j.bbrc.2009.06.142

Rose EP, Osterberg VR, Banga JS, Gorbunova V, Unni VK. 2024. Alpha-synuclein regulates the repair of genomic DNA double-strand breaks in a DNA-$PK_{cs}$-dependent manner. : 10.1101/2024.02.29.582819. DOI: 10.1101/2024.02.29.582819

Samuel F, Flavin WP, Iqbal S, Pacelli C, Sri Renganathan SD, Trudeau LE, et al., Tandon. 2016. Effects of Serine 129 Phosphorylation on α-Synuclein Aggregation, Membrane Association, and Internalization. Journal of Biological Chemistry 291: 4374-4385. DOI: 10.1074/jbc.M115.705095

Schaser AJ, Osterberg VR, Dent SE, Stackhouse TL, Wakeham CM, Boutros SW, et al., Unni. 2019. Alpha-synuclein is a DNA binding protein that modulates DNA repair with implications for Lewy body disorders. Scientific Reports 9: 10.1038/s41598-019-47227-z. DOI: 10.1038/s41598-019-47227-z

Schaser AJ, Stackhouse TL, Weston LJ, Kerstein PC, Osterberg VR, López CS, et al., Unni. 2020. Trans-synaptic and retrograde axonal spread of Lewy pathology following pre-formed fibril injection in an in vivo A53T alpha-synuclein mouse model of synucleinopathy. Acta Neuropathologica Communications 8: 10.1186/s40478-020-01026-0. DOI: 10.1186/s40478-020-01026-0

Spinelli KJ, Taylor JK, Osterberg VR, Churchill MJ, Pollock E, Moore C, Meshul CK, Unni VK. 2014. Presynaptic Alpha-Synuclein Aggregation in a Mouse Model of Parkinson's Disease. The Journal of Neuroscience 34: 2037-2050. DOI: 10.1523/JNEUROSCI.2581-13.2014

Tenreiro S, Eckermann K, Outeiro TF. 2014. Protein phosphorylation in neurodegeneration: friend or foe?. Frontiers in Molecular Neuroscience 7: 10.3389/fnmol.2014.00042. DOI: 10.3389/fnmol.2014.00042

Wang H, Qiu Z, Liu B, Wu Y, Ren J, Liu Y, et al., Xu. 2018. PLK1 targets CtIP to promote microhomology-mediated end joining. Nucleic Acids Research : 10.1093/nar/gky810. DOI: 10.1093/nar/gky810

Waxman EA, Giasson BI. 2010. Characterization of kinases involved in the phosphorylation of aggregated α-synuclein. Journal of Neuroscience Research 89: 231-247. DOI: 10.1002/jnr.22537

Weston LJ, Stackhouse TL, Spinelli KJ, Boutros SW, Rose EP, Osterberg VR, et al., Unni. 2021. Genetic deletion of Polo-like kinase 2 reduces alpha-synuclein serine-129 phosphorylation in presynaptic terminals but not Lewy bodies. Journal of Biological Chemistry 296: 100273. DOI: 10.1016/j.jbc.2021.100273

Wu W, Sung CC, Yu P, Li J, Chung KKK. 2020. S-Nitrosylation of G protein-coupled receptor kinase 6 and Casein kinase 2 alpha modulates their kinase activity toward alpha-synuclein phosphorylation in an animal model of Parkinson’s disease. PLOS ONE 15: e0232019. DOI: 10.1371/journal.pone.0232019

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