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Proximity Ligation Assay (PLA)

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We use this protocol and it's working

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Abstract

Proximity Ligation Assay (PLA)

1 The PLA assay was performed as described previously with minor modifications [48]. The following antibodies were used for the PLA experiments: Syn 204 against h-αSyn (Santa Cruz Biotechnology Cat# sc-32280, RRID:AB_628319)(1:100) and EPR12790 against VAMP-2 (Abcam Cat# ab214590)(1:300).

2 The *in-situ* PLA was performed on fixed primary neurons with DuoLink PLA technology probes and reagents (Sigma-Aldrich Cat# DUO92002, DUO92004, DUO82049, DUO92008, and DUO92014), following the manufacturer's protocol. First, the neurons were permeabilized with PBS + 0.4% Triton X-100 for 10 min.

3 After two PBS washes, the cells were incubated with a blocking solution for 2 hours at 37 °C and then incubated with the primary antibodies for 30 min at room temperature.

4 The coverslips were washed twice for 5 min with buffer A, followed by incubation with the PLA probes (secondary antibodies against two different species bound to two oligonucleotides: anti-mouse MINUS (Sigma-Aldrich Cat# DUO92004 (also DUO92004-30RXN, DUO92004-100RXN), RRID:AB_2713942) and anti-rabbit PLUS) (Bethyl Cat# OLK-92002-0100, RRID:AB_10950581)in antibody diluent for 30 min at 37 °C. After two washes of 5 min with buffer A, the ligation step was performed with ligase diluted in ligation stock for 30 min at 37 °C.

5 The coverslips were washed with buffer A twice for 2 min before incubation for 50 min with amplification stock solution at 37 °C. After two washes of 10 min with buffer B. Finally, the coverslips were washed with PBS and mounted with Duolink *in situ* mounting medium (Sigma-Aldrich Cat# DUO82040-5 ML).

6 A negative control experiment was performed for every antibody, where only one antibody was incubated with the PLA probes. The experiments were performed 2 times. The experiments were performed 2 times. Average signal intensities were measured using the MetaMorph Microscopy Automation and Image Analysis Software (RRID:SCR_002368) (https://www.moleculardevices.com/products/cellular-

imaging-systems/acquisition-and-analysis-software/metamorph-microscopy#gref) and plotted using GraphPad Prism
software [(RRID:SCR_002798) http://www.graphpad.com/].