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We use this protocol and it's working

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Immunofluorescence and object-based colocalization analysis

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ABSTRACT

Immunofluorescence and object-based colocalization analysis

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Immunofluorescence

1

For immunostaining, DIV17-21 neurons were fixed in 100% ice-cold methanol solution for 15 min at room temperature, followed by extraction in 0.1 % Triton X-100 for 10 min, blocked in 10% BSA for 1 h at room temperature, and incubated overnight at 4°C with primary antibodies. We found that the methanol fixation was better than paraformaldehyde fixation for pSer129 α-syn staining.

2 After washing with 1x PBS, neurons were blocked again for 30 min at room temperature and incubated with secondary antibodies (Alexa Fluor antibodies from Invitrogen (RRID:AB_2633275, RRID:AB_2762824,RRID:AB_2633282) (1:500) for 1 h at room temperature.

3 Images were acquired at 40X magnification. Z-stack images were obtained as previously described , and all images were acquired and processed 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).

Object-based colocalization analysis

4 For α-syn Ser129 and VAMP2 colocalization analysis in cultured neurons, 5-6 regions of interest (ROIs) of 200x200 pixels were placed on each image. A total of 26 ROIs were used for this analysis.

5 Object-based colocalization analysis was done

using MATLAB (RRID:SCR_001622)(http://www.mathworks.com/products/matlab/). First, automatic puncta detection was done using local maxima. Next, detected puncta are said to have co-localized if the distance between their centers is less than the maximum radius of the two particles.