Supplemental information

Kabuta et al.
Title: Aberrant interaction between Parkinson's disease-associated mutant UCH-L1 and the lysosomal receptor for chaperone-mediated autophagy

Experimental Procedures

Plasmids. The expression plasmids pCI-neo-hLAMP-2A, containing human WT LAMP-2A, and pCI-neo-hLAMP-2AΔC, containing LAMP-2A in which C-terminal cytosolic region was substituted by HA tag, were constructed using the pCI-neo mammalian expression vector.

siRNA preparation and transfection. A double-stranded short interfering RNA (siRNA) targeting LAMP-2 was purchased from RNAi Co., Ltd. (Tokyo, Japan). Sequences targeted by siRNA were selected using siDirect (RNAi Co., Ltd): sense (5'-CUUAUAUGUGCAACAAAGAGC-3') and antisense (5'-UCUUUGUUGCACAUAUAAGAA-3'). EGFP siRNA (1) was used as a control. Cells were transfected with siRNA using X-tremeGENE siRNA Transfection Reagent (Roche Diagnostics).

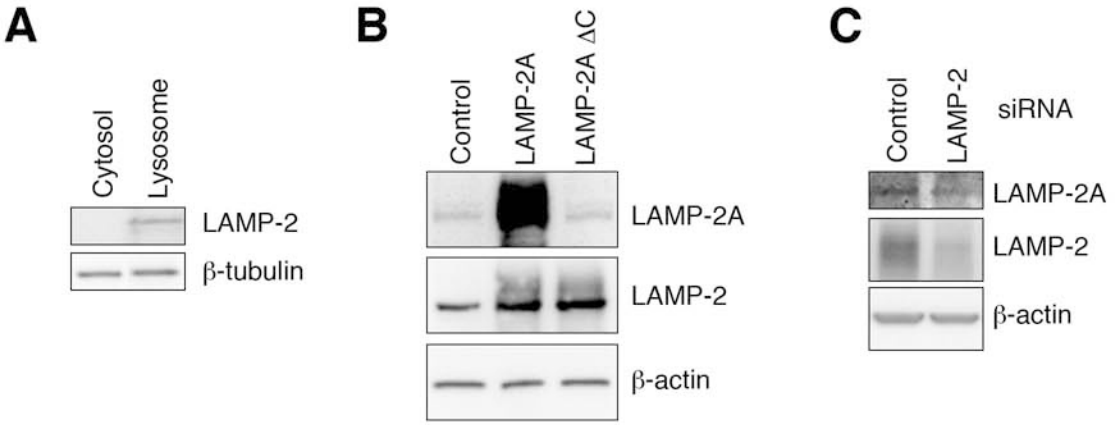
REFERENCES

1. Kabuta, T., Suzuki, Y., and Wada, K. (2006) *J. Biol. Chem.* **281**, 30524-30533

FIGURE LEGENDS

Figure S1 (A) Cytosolic and lysosomal fractions were prepared and immunoblotted with anti-LAMP-2 and β-tubulin antibodies. **(B, C)** Specificity of the anti-LAMP-2A antibody. COS-7 cells were transfected with the indicated constructs (control: empty vector) (B). Cell lysates were prepared and immunoblotted using anti-LAMP-2A, LAMP-2 and β-actin antibodies (B). COS-7 cells were transfected with the indicated siRNAs (C). Cell lysates were prepared and analyzed by immunoblotting using anti-LAMP-2A, LAMP-2 and β-actin antibodies (C).

Figure S2 (A) Lysates of COS-7 cells transfected with the indicated constructs (Control: empty vector) were immunoprecipitated with anti-FLAG antibody, and analyzed by immunoblotting. **(B)** COS-7 cells were transfected with the indicated constructs. Cell lysates were prepared and immunoblotted with anti-α-synuclein, UCH-L1 and β-actin antibodies.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β-tubulin
B
Control
LAMP-2A
LAMP-2A ΔC
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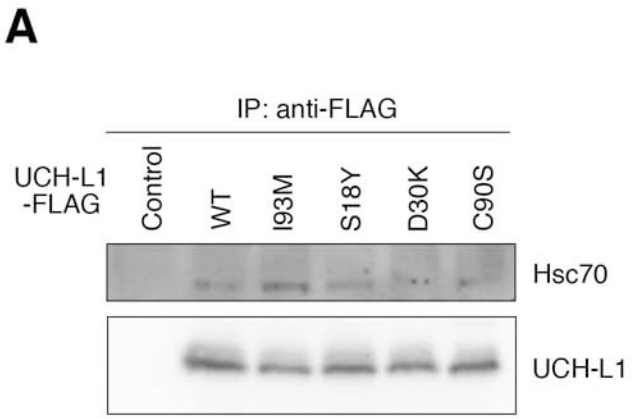
LAMP-2
β-actin
C
Control
LAMP-2
siRNA
LAMP-2A
LAMP-2
β-actin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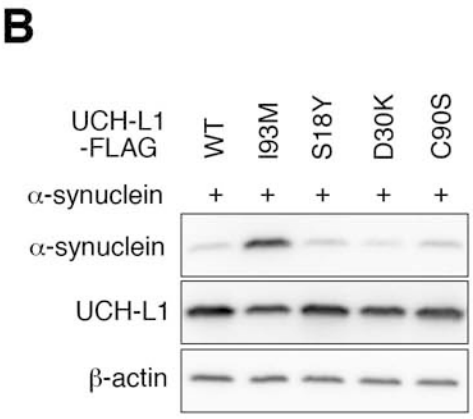
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UCH-L1
-FLAG
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α-synuclein
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β-actin