

## SUPPLEMENTARY MATERIALS

# Knockdown of PEX16 Induces Autophagic Degradation of Peroxisomes

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## **SUPPLEMENTARY METHOD**

### **Measurement of Reactive Oxygen Species (ROS)**

Intracellular ROS generation was detected using 2',7'-dichlorofluorescein diacetate (CM-H<sub>2</sub>DCFDA) (#C6827, Invitrogen, Eugene, OR, USA). RPE-1 cells were treated with *PEX16* siRNA for 72 h. DCFDA (1  $\mu$ M) was added to the cells, followed by incubation for 45 min at 37°C in a 5% CO<sub>2</sub> incubator. Cells were then fixed, and the fluorescence intensity was examined with an IX71 fluorescence microscope (Olympus, Tokyo, Japan).

### **Immunoprecipitation**

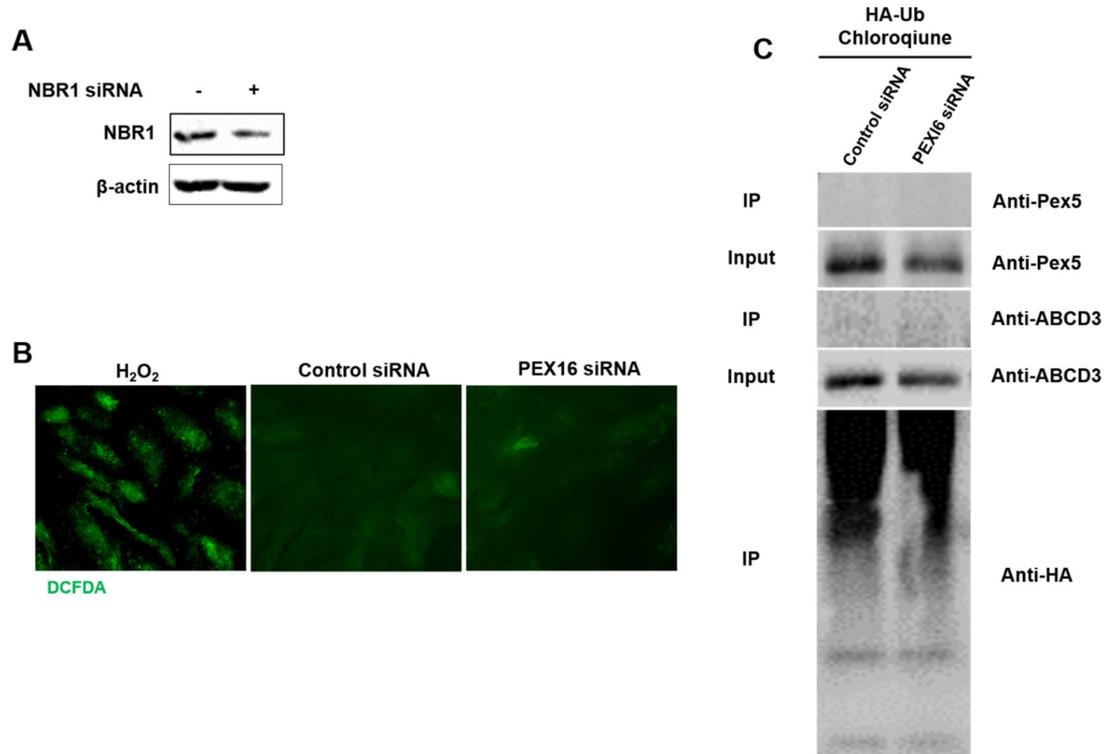
Immunoprecipitation was performed using Pierce Classic IP Kit (#26146, Pierce Biotechnology, Rockford, IL, USA) according to the manufacturer's instructions.

## SUPPLEMENTARY TABLE

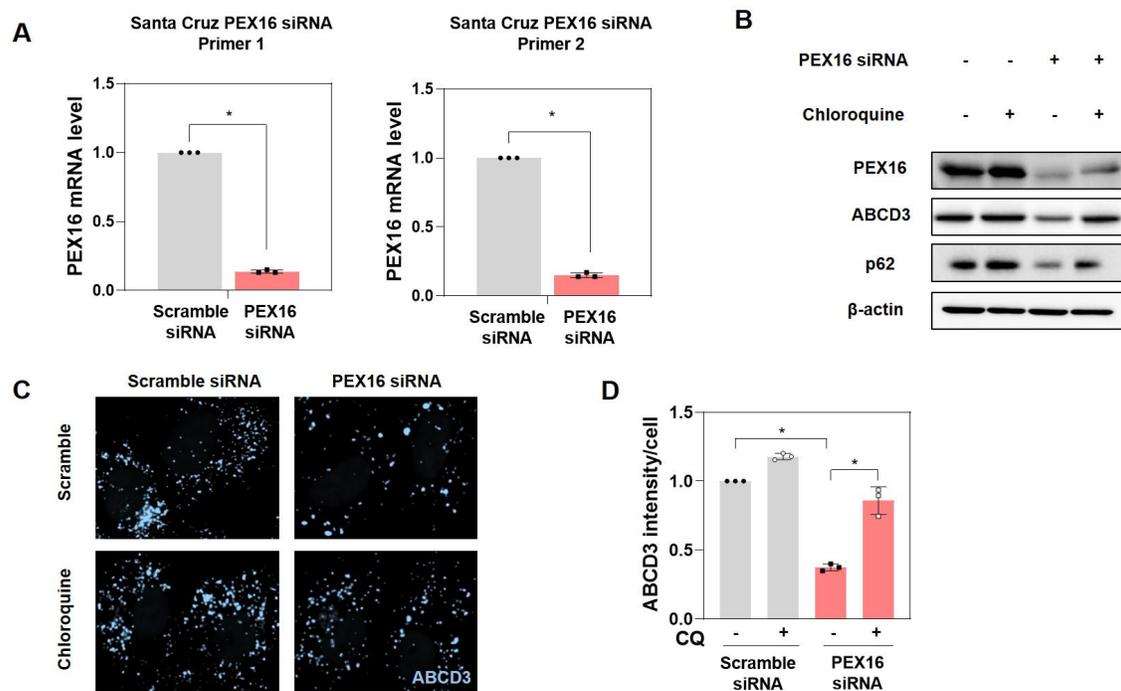
**Table S1. Primer sequences used in the present study**

<b>Genes</b>	<b>Forward</b>	<b>Reverse</b>
36B4	TGCATCAGTACCCCATTCTATCA	AAGGTGTAATCCGTCTCCACAGA
BECLIN1	AGGATGGTGTCTCTCGAAGATT	GATCAGAGTGAAGCTATTAGCATCTTC
GABARAP	GCGAGAAAATCCGAAAGAAA	AGATCAGAAGGCACCAGGTATT
LC3B	CGCACCTTCGAACAAAGAG	CTCACCTTGTATCGTTCTATTATCA
PEX16-1	CTGGTGTACTCTGCCTCTAAC	CATCTCCATGAACACCTCCA
PEX16-2	ACCATCCTGCTGCTCTACTA	TAATCCATGAGCGGCCTTG

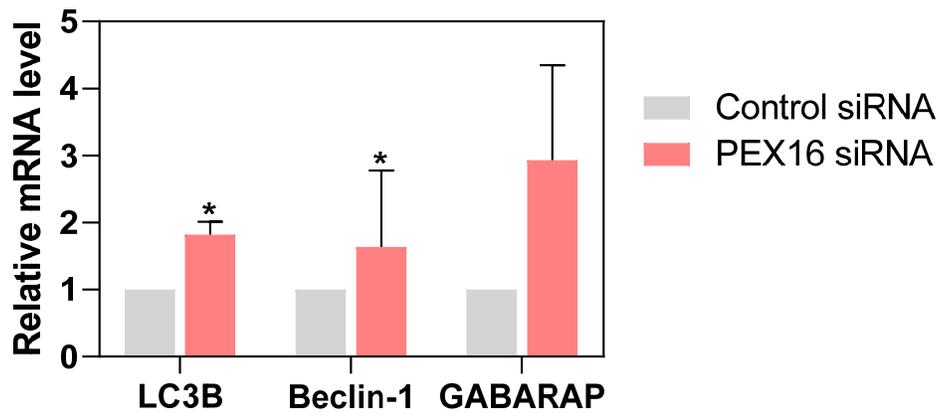
SUPPLEMENTARY FIGURES



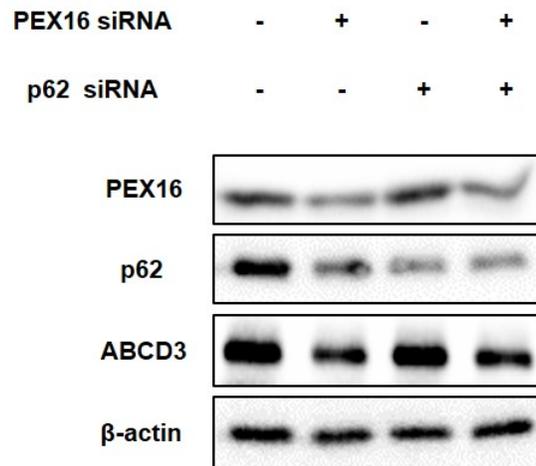
**Figure S1.** Pexophagy-induced by *PEX16* knockdown is not associated to ROS and PEX5 ubiquitination. **(A)** The immunoblot, confirming the transfection efficiency of NBR1. **(B)** The cellular ROS was detected using DCFDA. **(C)** RPE-1 cells were treated with *PEX16* siRNA for 48 h, transfected with HA-Ubiquitin plasmid in the presence of chloroquine for 24 h, then harvested and subjected to immunoprecipitation.



**Figure S2.** Inhibition of autophagy restores peroxisome abundance in RPE-1 cells with *PEX16* knockdown. **(A)** RPE-1 cells were transfected with siRNA of *PEX16* (Santa Cruz, #sc-96993) for 72 h. The transfection efficiency was evaluated by measuring the *PEX16* mRNA level with RT-qPCR. **(B)** Cells were treated with either Scramble siRNA or *PEX16* siRNA for 12 h, followed by incubation with 5  $\mu$ M chloroquine (CQ) for 60 h. The protein expression of PEX16, ABCD3, and p62 was examined with immunoblotting. **(C)** The ABCD3 expression was also analyzed using immunofluorescence staining. **(D)** Quantification of ABCD3 intensity from (C). All data are presented as means  $\pm$  S.D. (n = 3, independent experiments), \*p < 0.05.



**Figure S3.** The expression of autophagy-related genes in RPE-1 cells with *PEX16* knockdown. RPE-1 cells were treated with siRNA of either control or *PEX16* for 72 h, and the transcription level of autophagy related genes was analyzed with RT-qPCR.



**Figure S4.** ABCD3 expression is recovered in HEK293T cells with *PEX16* and *p62* knockdown. HEK293T cells were co-transfected with siRNA of p62 and PEX16, and the protein lysate was analyzed by immunoblotting to check the expression of PEX16, p62, and ABCD3.