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Supplemental Material to:

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The membrane peroxin PEX3 induces peroxisome-ubiquitination-linked pexophagy

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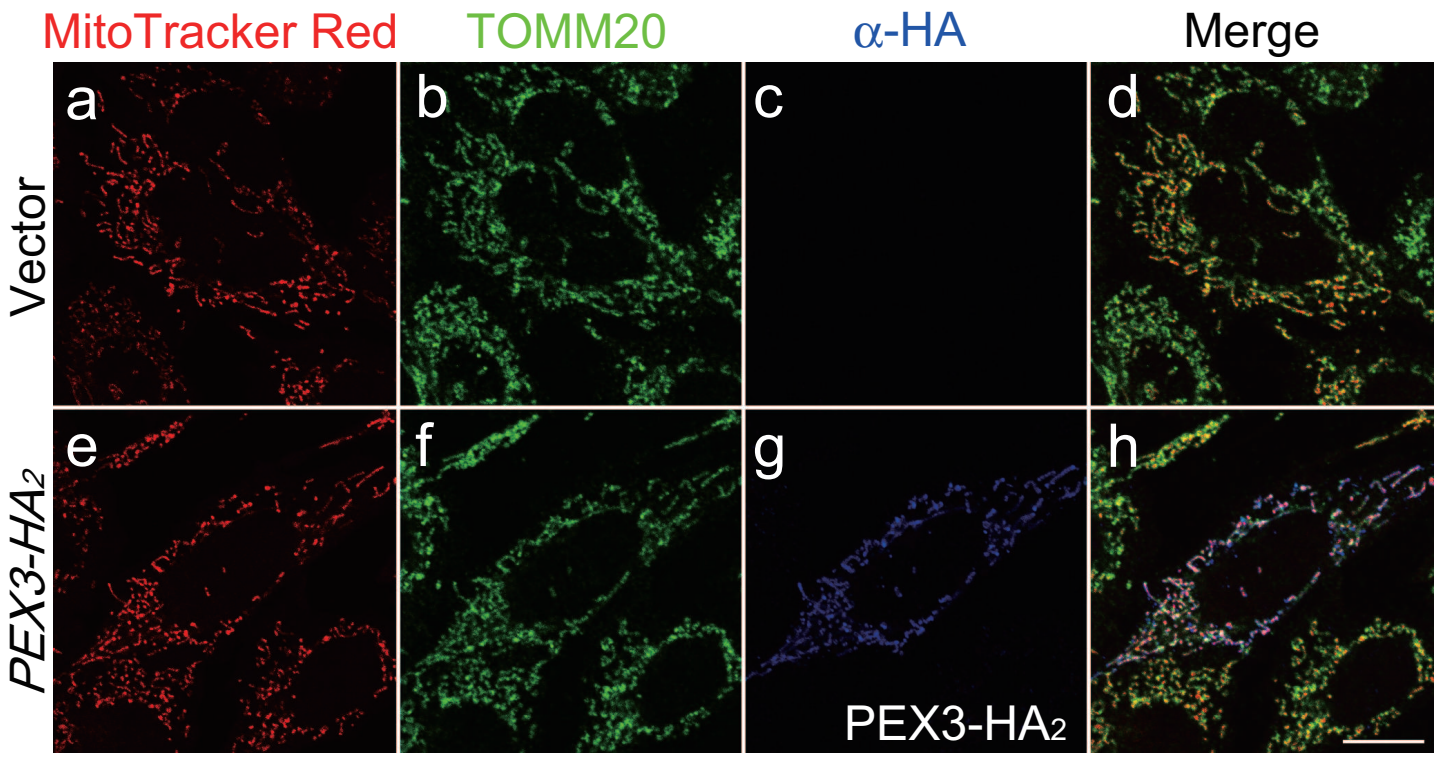
A CHO-K1



B CHO-K1

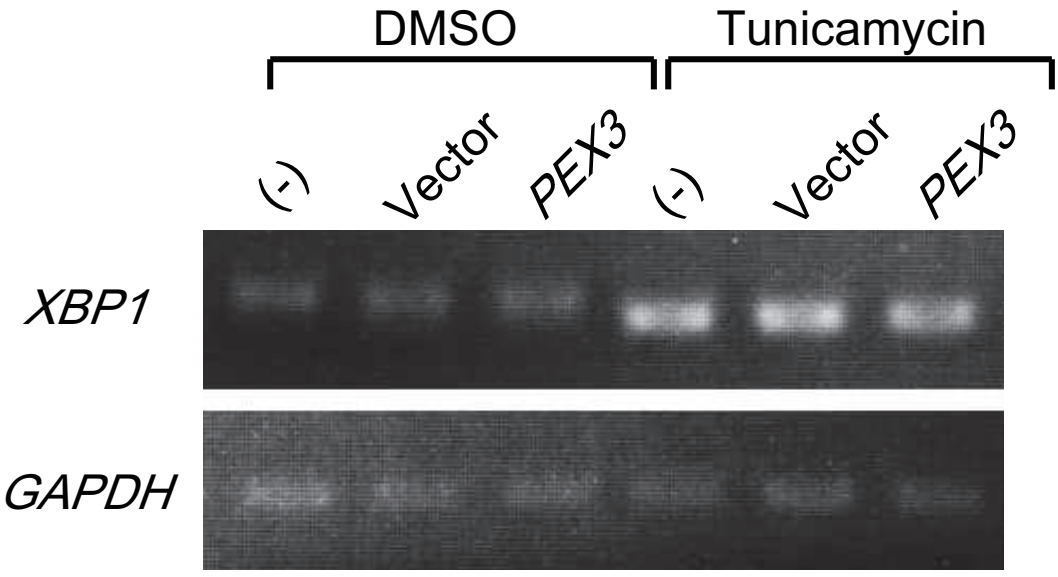
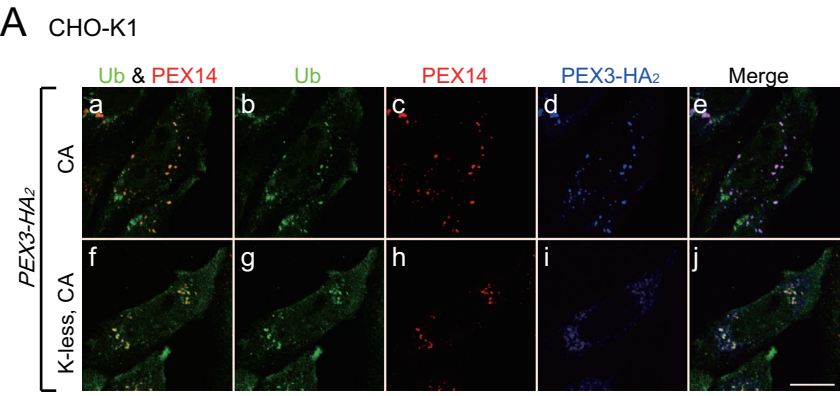


Figure S1


A
CHO-K1
Ub & PEX14
Ub
PEX14
PEX3-HA2
Merge
a
b
c
d
e
f
g
h
i
j
CA
K-less, CA
PEX3-HA2

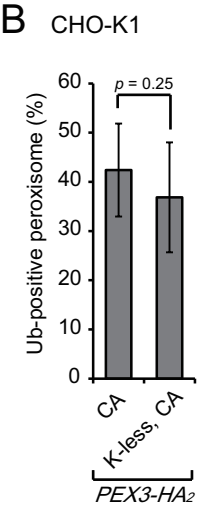

B
CHO-K1
p = 0.25
Ub-positive peroxisome (%)
60
50
40
30
20
10
0
CA
K-less, CA
PEX3-HA2

Figure S2

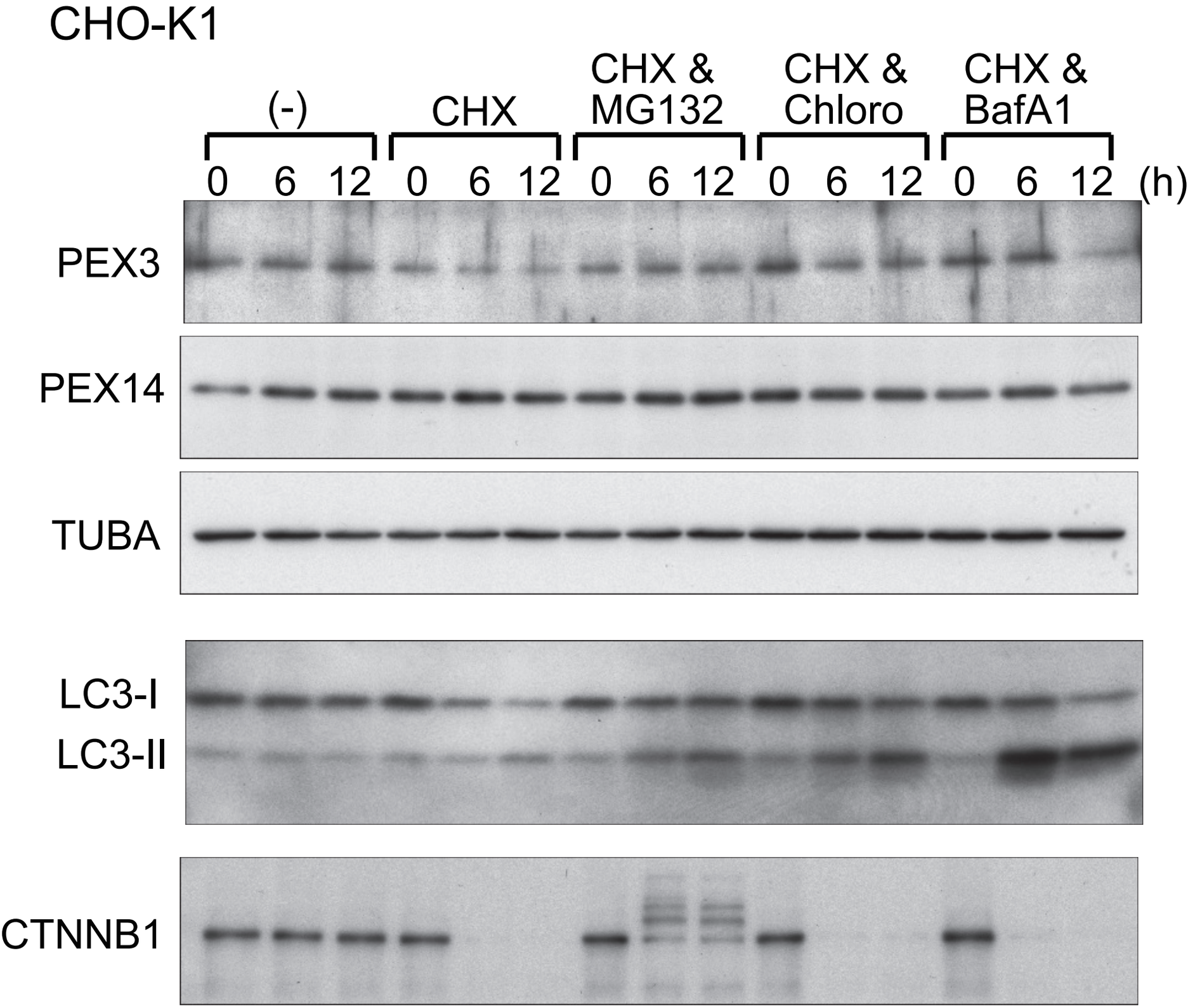

CHO-K1
(-)
CHX
CHX & MG132
CHX & Chloro
CHX & BafA1
0 6 12 0 6 12 0 6 12 0 6 12 0 6 12 (h)
PEX3
PEX14
TUBA
LC3-I
LC3-II
CTNNB1

Figure S3

Figure S1. PEX3 overexpression does not induce mitochondrial depolarization and ER stress. (**A**) CHO-K1 cells were transfected with empty vector or *PEX3-HA2*. After 24 h, the cells were stained with 50 nM MitoTracker Red (a and e) for 15 min, and then fixed and immunostained with antibodies against TOMM20 (b and f) and HA (c and g). Scale bar: 10 μm. (**B**) CHO-K1 cells were transfected with empty vector or *PEX3-HA2* in the presence of DMSO or tunicamycin. After 12 h, total RNA was isolated from the cells and subjected to reverse transcription (RT). To assess the splicing of *XBP1* in response to ER stress, *XBP1* cDNA was amplified from the RT products using specific primers (upper panel). *GAPDH* cDNA was also amplified as a control (lower panel).

Figure S2. Overexpression of PEX3-HA2 CA mutants induces peroxisomal ubiquitination. (**A**) CHO-K1 cells were transfected with *PEX3-HA2* CA or CA, K-less mutants. After 12 h, the cells fixed and immunostained with antibodies against ubiquitin (b and g), PEX14 (c and h) and HA (d and i). Merged view of ubiquitin and PEX14 are shown in a and f. Scale bar: 10 μm. (**B**) Percentages of peroxisome signals colocalized with ubiquitin were calculated with Metamorph version 7.6 software from ten cells.

Figure S3. PEX3 is constitutively degraded by the proteasome. CHO-K1 cells were incubated for 12 h in the presence of cycloheximide (CHX) with a proteasome inhibitor, MG132, and, lysosome inhibitors, chloroquine (Chloro) and bafilomycinA_1 (BafA1), as indicated at the top. The cells were lysed with lysis buffer containing 1% TritonX-100 at each 0, 6, and 12 h and analyzed by SDS-PAGE and immunoblotting with antibodies against PEX3, PEX14, and TUBA/α-tubulin. To assess the effect of inhibitors, LC3 (for lysosome activity) and CTNNB1/β-catenin (for protein synthesis and proteasome activity) were also analyzed.

Table S1. Sequences of siRNAs used in this study

Name	Strand	Sequence
Luciferase (a control) siRNA	Sense	5’-UUCACUGGCGACGUAAUCCACGAUC-3’
	Antisense	5’-GAUCGUGGAUUACGUCGCCAGUCAA-3’
Human p62 siRNA	Sense	5’-UACGUGAAGGAUGACAUCUUCCGAA-3’
	Antisense	5’-UUCGGAAGAUGUCAUCCUUCACGUA-3’
Human NBR1 siRNA	Sense	5’-UUUACCAUAGCUUCGAUAUCAGCCC-3’
	Antisense	5’-GGGCUGAUAUCGAAGCUAUGGUAAA-3’

Table S2. Oligonucleotide primers used in this study

Name	Sequence (5' to 3')
p62FwEco	GGAATTCGCGTCGTTCACGGTGAAGGCCTATC
p62RvXho	CCGCTCGAGTCACAATGGTGGAGGGTGCTTCG
NBR1FwNot	GCCGGCGGCCGCTCGAACCACAGGTTACTCTAAATGTGAC
NBR1RvXba	GCTCTAGATCAATAGCGGTGGCTGTACCAGTCGTTG
NBR1D50RFw	CTATCCAGATAAAATACCTGCGTGAGGAGAACGAGGAGATATC
NBR1D50RRv	GATATCTCCTCGTTCTCCTCACGCAGGTATTTTATCTGGATAG
NBR1DCCFw	CATTTATGGAATTCAATCCATGGACTTCAGAG
NBR1DCCRv	GAGCCTGACTTGTTCCAAAGCAGCAGGCAGACG
NBR1DLIR2Fw	GAAGAAACAGAAGAAGACCTGAGTGGGACCC
NBR1DLIR2Rv	TCTTGTCTTCTGTGAGGCACTGGCTCCTG
NBR1Y750AFw	CTGCTTCCTCTGAAGATGCTATCATCATCCTTCCTGAG
NBR1Y750ARv	CTCAGGAAGGATGATGATAGCATCTTCAGAGGAAGCAG
NBR1DJ-UBAFw	GTTTCTGAAGATCAGACCACAGCCCTGATGGCCC
NBR1DJ-UBARv	GTCACAGCTCCTCTGTCTGCTGTTTGCGAGTCC
NBR1DUBARv	GCTCTAGATCAGATGGGCTGTGCAGTGACTGGTGGCCCAG
NBR1DC-termRv	GCTCTAGATCATGGGGGAACTTCGTGCATGGTAGC
NBR1siRNA-resistFw	GGCAATGGTGAAAGTTTCATTTGATCTGAATACTATCCAGATAAAATACCTGGATG
NBR1siRNA-resistRv	TCAACGTCTGCCCAGGTTGTATTTTCTGGATCCGAAACCAGAAAGCTTTGAGTTTC

LC3BFwEco	GGAATTCCCGTCCGAGAAGACCTTCAAGCAGCG
LC3BRvXho	CCGCTCGAGTTACACAGCCATTGCTGTCCCGAATGTC
PEX3-K0-fragment1Fw	CGGGATCCGCCACCATGCTGAGATCAATGTGGAATTTTCTGAGACGTCACAGAAGGAGATG
PEX3-K0-fragment1Rv	CTAAGTCTTCTCTGTCCATATCTTCCCAGG
PEX3-K0-fragment2	CTTATTATCCTTAGATCCTCCCATATTTCCAGCCTGTTTGAAGGCCTGTTTCTCAAC
PEX3-K0-fragment3Fw	GGCAGAAATGGCACTAGCATCCTAGC
PEX3-K0-fragment3Rv	GAATGTCTAAGAGAAATACTTCCTAAGATCCTCTGCACAGCTTGTCTAATGACAG
PEX3-K0-fragment4Fw	GTCCCTTTTGGACTTGGAGCAGAGACTGAGAGAAATAAG
PEX3-K0-fragment4Rv	CATGTAGTGGCACAGGGAAGACCTGGATGCGTCTCTATC
PEX3-K0-fragment5Fw	CCACTATTAGACTTCTCAATGAGACAAG
PEX3-K0-fragment5Rv	GATTATCCTGGCTAAAGGGAGGCTGACACTGG
PEX3-K0-fragment6Fw	CGTGCAGGATCTGCTGATGATGGAGCAAGTGAGAGACTTTGC
PEX3-K0-fragment6Rv	CCGCTCGAGTCATCTCTCCAGTTGTTGGGGGGTG