Landes Bioscience www.landesbioscience.com

Supplemental Material to:

Ji Zhang, Melanie R. Loyd, Mindy S. Randall, M. Brett Waddell, Richard W. Kriwacki and Paul A. Ney

A short linear motif in BNIP3L (NIX) mediates mitochondrial clearance in reticulocytes

Autophagy 2012; 8(9)
http://dx.doi.org/10.4161/auto.20764

www.landesbioscience.com/journals/autophagy/article/20764

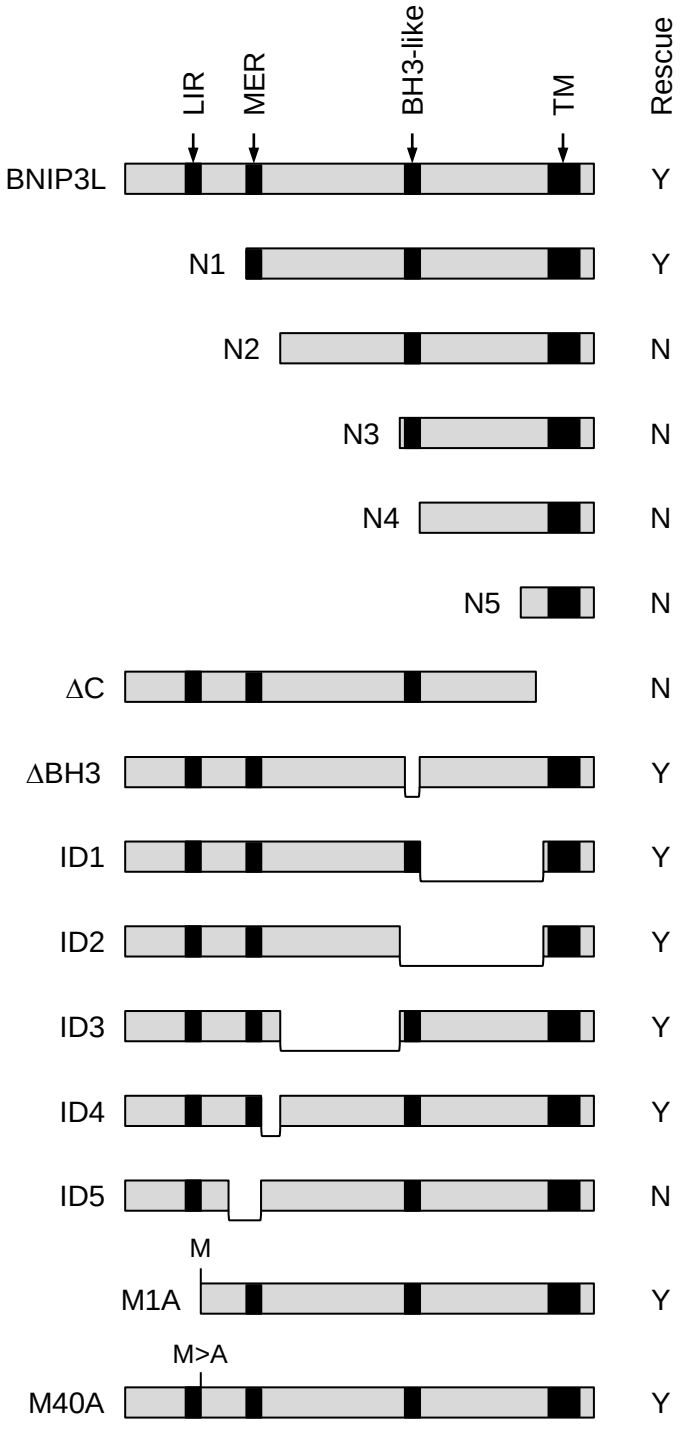

LIR
MER
BH3-like
TM
Rescue
BNIP3L
Y
N1
Y
N2
N
N3
N
N4
N
N5
N
ΔC
N
ΔBH3
Y
ID1
Y
ID2
Y
ID3
Y
ID4
Y
ID5
N
M
M1A
Y
M>A
M40A
Y

Figure S2

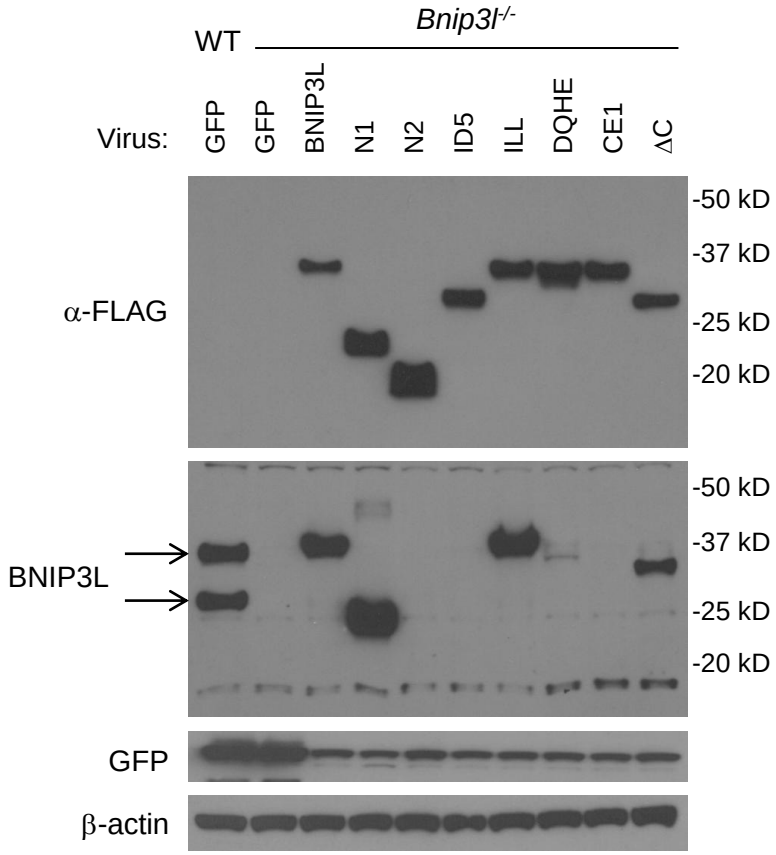

WT
Bnip3l-/-
Virus:
GFP
GFP
BNIP3L
N1
N2
ID5
ILL
DQHE
CE1
ΔC
α-FLAG
-50 kD
-37 kD
-25 kD
-20 kD
BNIP3L
-50 kD
-37 kD
-25 kD
-20 kD
GFP
β-actin

Figure S3

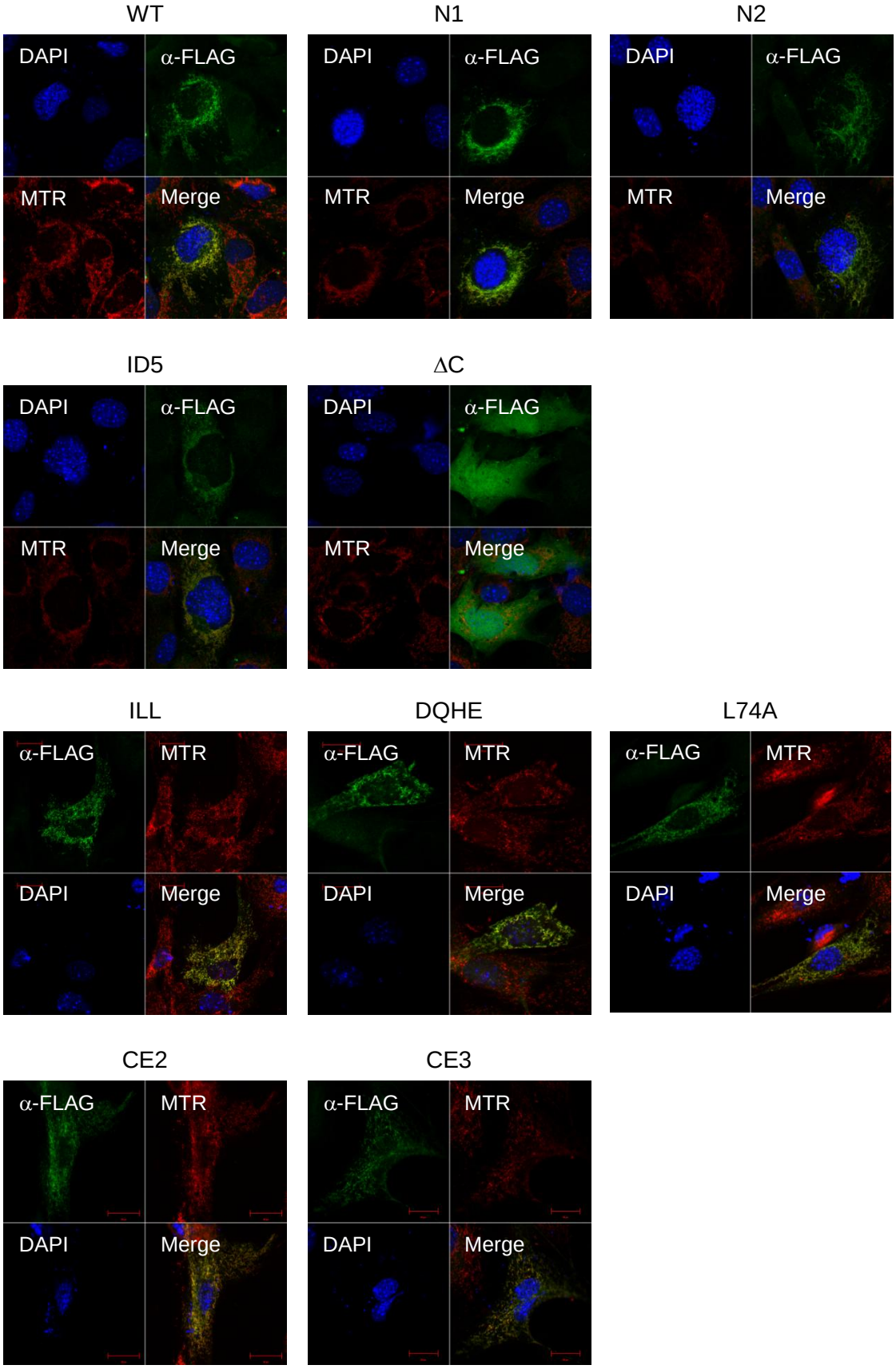
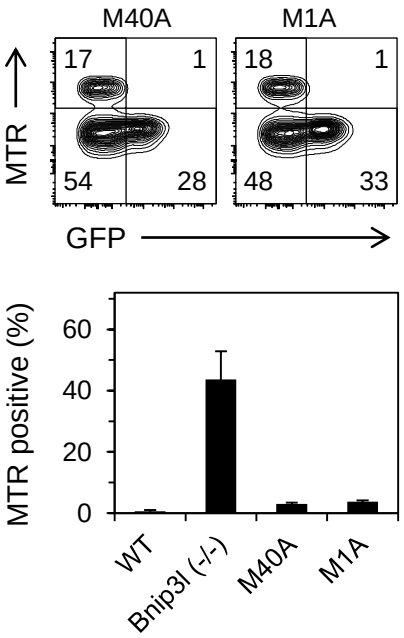

WT
DAPI
α-FLAG
MTR
Merge
N1
DAPI
α-FLAG
MTR
Merge
N2
DAPI
α-FLAG
MTR
Merge
ID5
DAPI
α-FLAG
MTR
Merge
ΔC
DAPI
α-FLAG
MTR
Merge
ILL
α-FLAG
MTR
DAPI
Merge
DQHE
α-FLAG
MTR
DAPI
Merge
L74A
α-FLAG
MTR
DAPI
Merge
CE2
α-FLAG
MTR
DAPI
Merge
CE3
α-FLAG
MTR
DAPI
Merge

Figure S4


M40A
M1A
17
1
54
28
18
1
48
33
MTR
GFP
MTR positive (%)
60
40
20
0
WT
Bnip3l (-/-)
M40A
M1A

Supplemental information

Figure legends

Figure S1. Schematic diagram of BNIP3L mutants. LIR, MER, BH3-like, and transmembrane (TM) domains are represented by black boxes. Deletions are represented by square brackets. Mutants are named on the left. Functional rescue of mitochondrial clearance is indicated on the right. The M1A BNIP3L mutant initiates from M40 but not M1. The M40A BNIP3L mutant initiates from M1 but not M40.

Figure S2. Expression of BNIP3L mutants. Embryonic day 14.5 *Bnip3l*$^{-/-}$ fetal liver cells were transduced with empty vector (GFP), BNIP3L, and BNIP3L mutant viruses. The first lane shows nontransduced wild type fetal liver cells for comparison. Extracts were probed with BNIP3L antibody or α-FLAG antibody. Of note, BNIP3L mutants N2, ID3, DQHE, and CE1 disrupt the epitope recognized by the BNIP3L antibody. Expression of these mutants, relative to endogenous, is determined by α-FLAG antibody and comparison with wild type FLAG-BNIP3L, which is recognized by both antibodies.

Figure S3. Subcellular localization of mutants. *Bnip3l*$^{-/-}$ murine embryonic fibroblasts were transduced with BNIP3L and BNIP3L mutant viruses. Fibroblasts were stained with MTR, fixed, permeabilized, stained with fluorescein-tagged α-FLAG antibody, and co-stained with DAPI. Merged images show colocalization of MTR-stained mitochondria and FLAG-BNIP3L mutants, except for the ΔC mutant, which lacks a transmembrane domain and exhibits diffuse cyto-plasmic staining.

Figure S4. The short and full-length forms of BNIP3L are fully active (and neither is uniquely required). Two BNIP3L mutants were examined for activity. Mutant M40A contains a methionine to alanine substitution at amino acid 40, and only expresses full-length BNIP3L. Mutant M1A expresses only the short form of BNIP3L (which does not include the LIR). Both forms contain the MER, and are active.