

Appendix

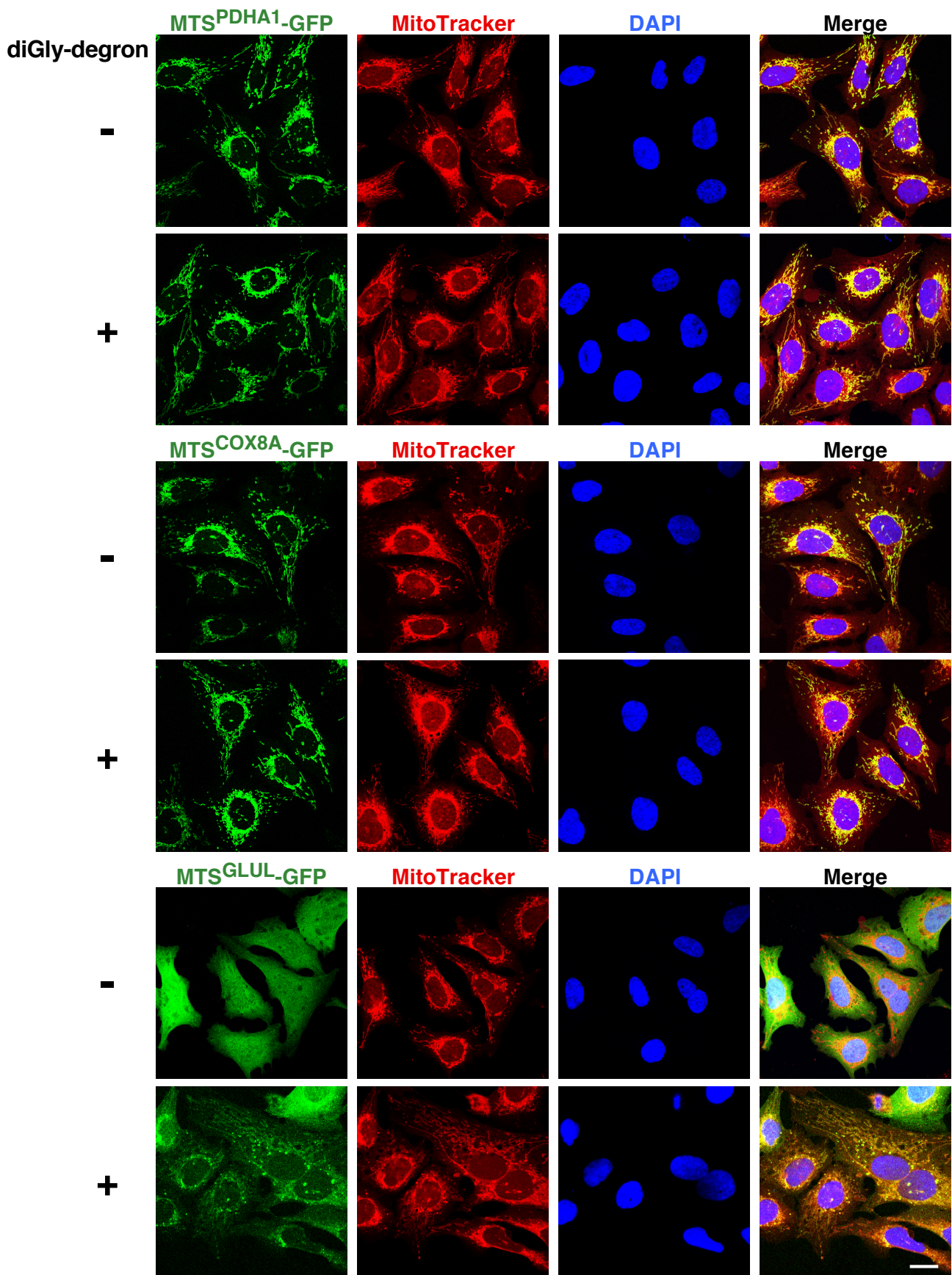
for

**The C-degron pathway eliminates mislocalized proteins
and products of deubiquitinating enzymes**

Yeh et al.

Content

Appendix Figure S1: Confocal images of U2OS cells expressing indicated MTS-GFP reporters with or without a diGly degron.	3
Appendix Figure S2: Amino acid sequence alignment of MIC19 from various species.	4
Appendix Figure S3: Live-cell images of indicated GFP fusion proteins in U2OS cells with or without DNCul2 or IMP-1088 treatment.	5
Appendix Figure S4: Confocal immunofluorescence images of U2OS cells stained with MitoTracker, DAPI and a MIC19 antibody (Abcam, ab224565) under the same condition applied to Figs. 5F, 6A, EV4I and EV4J.	6



Appendix Figure S1: Confocal images of U2OS cells expressing indicated MTS-GFP reporters with or without a diGly degron.

MitoTracker and DAPI staining indicate mitochondria and nucleus, respectively. Scale bar = 20 μm .

```

Human      1  MGGTTSRRRVTFEADENENITVVKGIRLSENVIDRMKE--SSPSG-SKSORYSGAYG-----
Chimpanzee 1  MGGTTSRRRVTFEADENENITVVKGIRLSENVIDRMKE--SSPSG-SKSORYSGAYG-----
Bovine     1  MGGTASRRRVTFEADENENITVVKGIRLSENVIDRMKE--SSPSG-SKSORYSGTYG-----
Mouse      1  MGGTASRRRVTFEADENENITVVKGIRLSENVIDRMKE--SSPSG-SKSORYSSVYG-----
Rat        1  MGGTASRRRVTFEADENENITVVKGIRLSENVIDRMKE--SSPSG-SKSORYSSVYG-----
Chicken    1  MGASGSTRRVTFEADENENITVVKGIRLSENVIDRMKEPSSPSGRPQSQRSP-----
Zebrafish  1  MGANNSTRRVTFEADENENITVVKGIRLSENVIDRMREPSCGFTS-PQVPSRPPAPPPVVDPLPALVQYLLHLTLSLFHPPPSLPERVKLPEQV
      *

Human      55  -----ASVDEELKRRVAEEL--ALEQAKKESDQKRL-----KQAKELDRERAAANEOLT
Chimpanzee 55  -----ASVDEELKRRVAEEL--ALEQAKKESDQKRL-----KQAKELDRERAAANEOLT
Bovine     55  -----ASVDEELKRRVAEEL--ALEQAKKESDQKRL-----KQAKELDRERAAANEOLT
Mouse      55  -----ASVDEELKRRVAEEL--ALEQAKKESDQKRL-----KQAKELDRERAAANEOLT
Rat        55  -----ASVDEELKRRVAEEL--ALEQAKKESDQKRL-----KQAKELDRERAAANEOLT
Chicken    54  -----GAVNDEELKRRVAEEL--ALEQAKKESDQKRL-----KQAKELDRERAAANEOLT
Zebrafish  95  STPAPPPPPPPPLESVGLSTPAVDSEELRKRKITEELRKGLEEDRRKTOEELNQWLEKEKSOAHAKAQAEQAQVKDEVSRILALEKSGAEETIQ

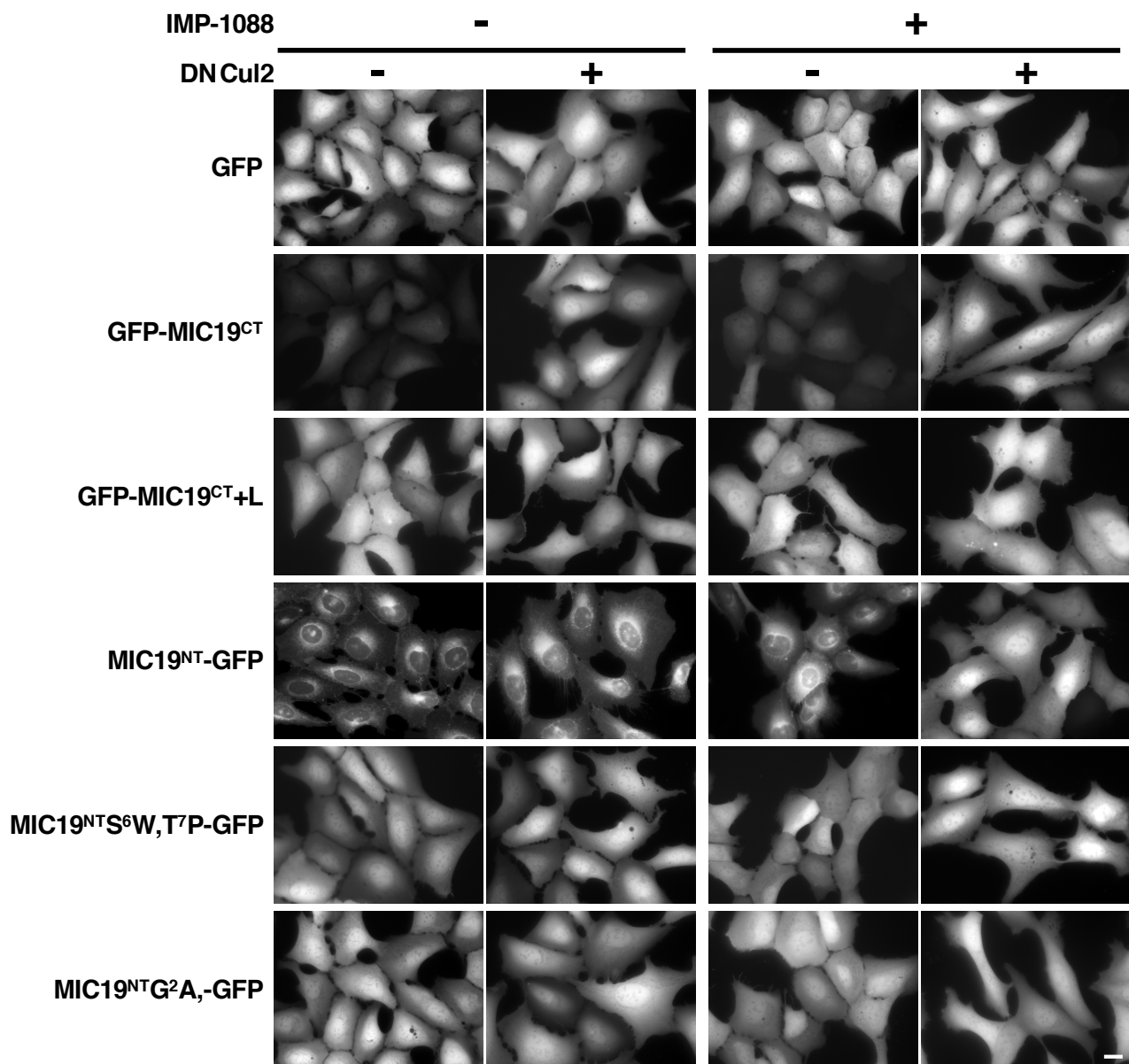
Human      104  RAILRERTCSEEEERAKAKHL-----AROLEEKDRVLKKODAFYKEQLARLEERSSEFYRVVTTEQYQKAAEVEEAKFKRYESHVPCADLQAKILQC
Chimpanzee 104  RAILRERISSEEEERAKAKHLDIEDKAROLEEKDRVLKKODAFYKEQLARLEERSSEFYRVVTTEQYQKAAEVEEAKFKRYESHVPCADLQAKILQC
Bovine     104  RAILRERISNEEEERAKAKHL-----AROLEEKDRVLKKODAFYKEQLARLEERSSEFYKVTTEQYQKAAEVEEAKFKRYESHVPCADLQAKILQC
Mouse      104  RAILRERISSEEEERAKAKHL-----AROLEEKDRVLKKODAFYKEQLARLEERSSEFYKVTTEQYQKAAEVEEAKFKRYESHVPCADLQAKILQC
Rat        104  RAILRERISSEEEERAKAKHL-----AROLEEKDRVLKKODAFYKEQLARLEERSSEFYKVTTEQYQKAAEVEEAKFKRYESHVPCADLQAKILQC
Chicken    113  RAILRERAAATEEEERAKAKHL-----AROLEEKDRVLKKODAFYKEQLARLEERSSEFYKVTTEQYQKAAEVEEAKFKRYESHVPCADLQAKILQC
Zebrafish  190  RAILRERISSEEEERAKAKHL-----AROLEEKDRVLKKODAFYKEQLARLEERSSEFYKVTTEQYQKAAEVEEAKFKRYESHVPCADLQAKILQC

Human      194  YRENTHQTLKCSALATQYMHCVNHAK-OSMLEKGG
Chimpanzee 199  YRENTHQTLKCSALATQYMHCVNHAK-OSMLEKGG
Bovine     194  YRONTQOTLSCSALASQYMRVCVNQAK-OSTLEKGG
Mouse      194  YRONTQOTLSCSALASQYMHCVNHAK-OSMLEKGG
Rat        194  YRONTQOTLSCSALANQYMHCVNHAK-OSMLEKGG
Chicken    203  YQOHAQETLSCSALANQYMHCVNHAK-OSMLEGRGG
Zebrafish  285  YRENAGKTLKCSALASQYMHCVNHAK-OSMLEKGG

```

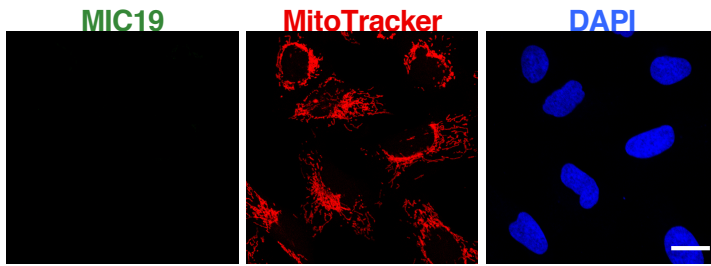
Appendix Figure S2: Amino acid sequence alignment of MIC19 from human (*Homo sapiens*), chimpanzee (*Pan troglodytes*), bovine (*Bos taurus*), mouse (*Mus musculus*), rat (*Rattus norvegicus*), chicken (*Gallus gallus*) and zebrafish (*Danio rerio*).

The myristoylation site is highlighted with an asterisk, and the C-terminal diGLy motif is shown in the box. The CHCH domain is underlined.



Appendix Figure S3: Live-cell images of indicated GFP fusion proteins in U2OS cells with or without DNCuI2 or IMP-1088 treatment.

Scale bar = 20 μ m.



Appendix Figure S4: Confocal immunofluorescence images of U2OS cells stained with MitoTracker, DAPI and a MIC19 antibody (Abcam, ab224565) under the same condition applied to Figs. 5F, 6A, EV4A and EV4C.

Scale bar = 20 μm .