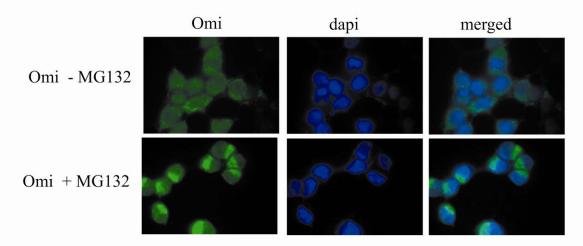


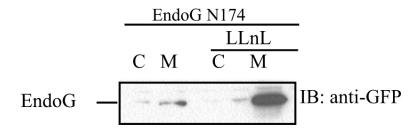
Supplemental Fig. S1: Inhibition of the proteasome promotes accumulation of cytochrome c. HEK293T cells were treated with or without the proteasome inhibitor LLnL or MG132 for 18 hours and the levels of endogenous cytochrome c determined by immunofluorescence.

Supplementary Fig. S2



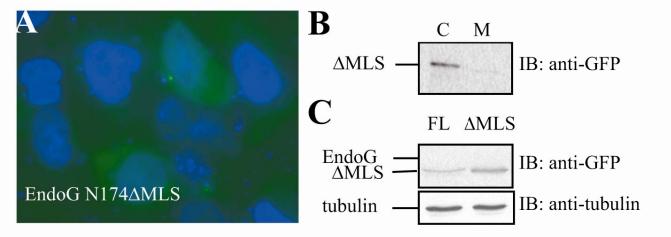
Supplemental Fig. S2: Inhibition of the proteasome promotes the accumulation of Omi . HEK293T cells were treated with or without the proteasome inhibitor MG132 for 18 hours and the levels of endogenous Omi determined by immunofluorescence.

Supplemental Fig. S3

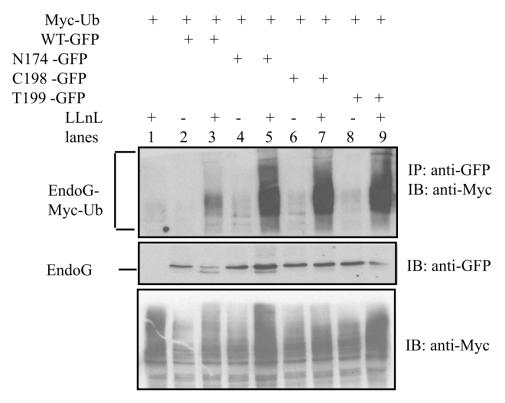


<u>Supplemental Fig. S3</u>: Inhibition of the proteasome promotes the accumulation of mutant EndoG in the mitochondrial fraction. HEK293T cells were transfected with mutant endoGN174 for 24 hours and treated with or without the proteasome inhibitor LLnL for an additional 18 hours. Cells were then fractionated into cytosolic (C) or mitochondrial (M) fractions and the levels of endoGN174 determined by immunoblot.

Supplemental Fig. S4



Supplemental Fig. S4: Deletion of the mitochondrial localization signal abolishes mitochondrial clustering. A) HEK293T cells were transfected with GFP-endoG-N174ΔMLS for 24 hours and cells were stained with dapi and visalized by immunofluorsence. B) HEK293T cells were transfected with GFP-endoG-N174ΔMLS for 24 hours and cells fractionated into cytosolic (C) or mitochondrial (M) fractions. The level of GFP-endoG-N174ΔMLS in each fraction was then determined by immunoblot. C) HEK293T cells were transfected with full lenght (FL) GFP-endoG-N174 or GFP-endoG-N174ΔMLS for 24 hours and the level of full lenght or ΔMLS-GFP-endoG determined by immunoblot on total proteins extract.



Supplemental <u>Fig. 5</u>: Mutants endoG are preferentially ubiquitinated compared to wild type endoG. HEK293T cells were transfected with indicated plasmids and the proteasome inhibitor LLnL added 18 hours before harvesting. GFP-endoG was immunoprecipiated (IP) using anti-GFP antibody and the immunoblot (IB) developed using anti-Myc to detected ubiquitinated endoG.