ESCRT-III-driven piecemeal *micro*-ER-phagy remodels the ER during recovery from ER stress

Marisa Loi et al.

Supplementary Information

Supplementary Figure 1



Supplementary Figure 1. A Endogenous SEC62 is delivered inside LAMP1 and GFP-RAB7 positive EL during recovery from CPA-induced ER stress in WT MEF exposed to 50 nM BafA1 for 12 h. **B** same as **A** when recov-ER-phagy is mimicked on overexpression of recombinant SEC62-HA in WT MEF exposed to BafA1 for 12 h. **C-G** Recov-ER-phagy mimicked on SEC62-HA overexpression in WT MEF and in *Atg4B-*, *Ulk1/2-*, *Atg13-* and *Atg14*KO MEF exposed to BafA1 for 12 h. Data are representative of at least three independent experiments. Scale bars: 10 μm.



Supplementary Figure 2. Control of p62 turnover (upper panel) and LC3 lipidation (middle panel) in the WT MEF and in MEF KO used in this study. GAPDH and actin as loading controls. Molecular weight markers are in kDa. Data shown in this figure confirm the results published by the groups sharing these cell lines (cited in the text and in the Acknowledgements section).



Supplementary Figure 3. A Delivery of SEC62-HaloTag within LAMP1 positive EL in WT MEF exposed to 50 nM BafA1 for 12 h. B SEC62LIR-HaloTag is not delivered within the EL. Data are representative of at least three independent experiments. Scale bars: 10 µm. C Translocation assay showing compromised ERj3 translocation (lane 5) in HEK CRISPR62 cells and confirming the functionality of SEC62-HaloTag and SEC62LIR-HaloTag in reestablishing ERj3 translocation (lane 6-9) in CRISPR62 cells. Molecular weight markers in WB are in kDa. WB is representative of at least three independent experiments (please also refer to ⁷). Mature (m)ERj3 has slower electrophoretic mobility than pre-(p)ERJ3 due to Nglycosylation that only occurs when the protein is translocated within the ER.















Supplementary Figure 4 - Uncropped Western blots.



Figure 5A





Supplementary Figure 2







Ulk1/2DKO

66-

14.5-

45-



Atg13KO





GAPDH

LC3

p62

Supplementary Figure 3C

