Supplemental material

Centonze et al., https://doi.org/10.1083/jcb.201903068

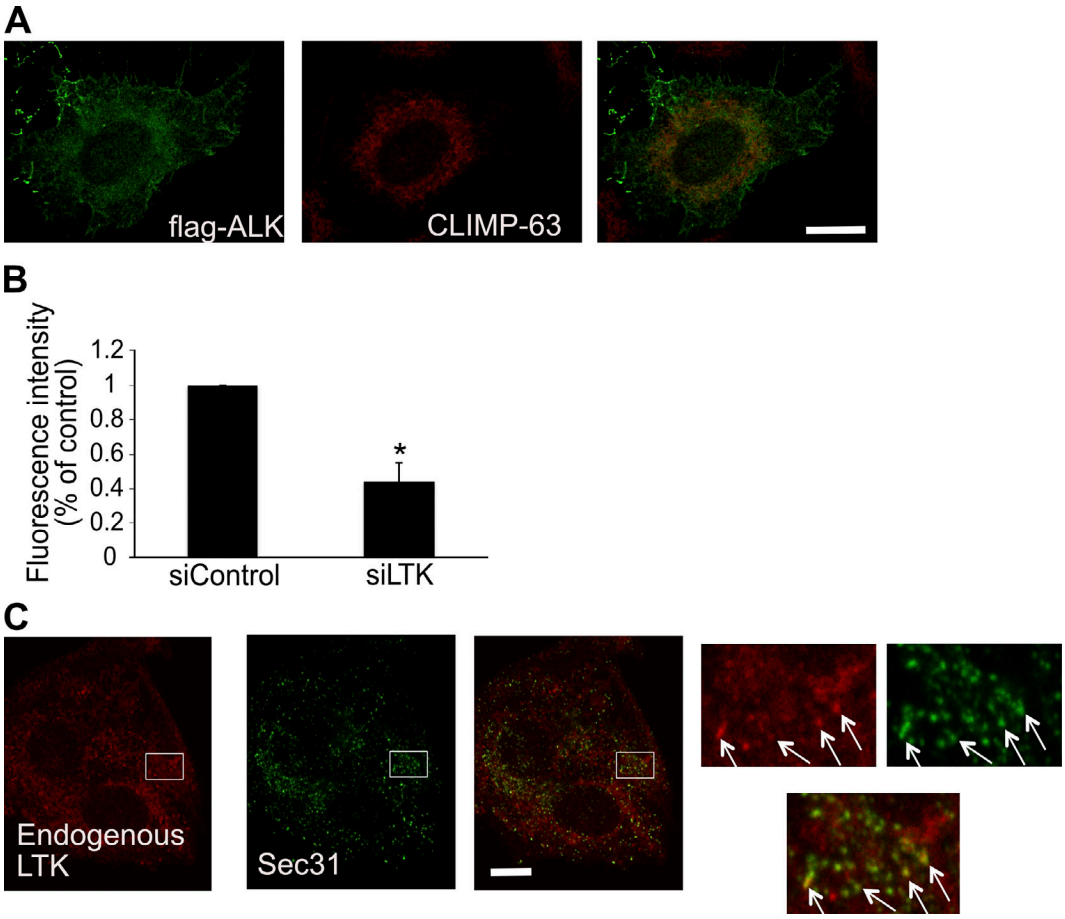


Figure S1. **Subcellular localization of ALK and LTK. (A)** HeLa cells expressing flag-tagged ALK were fixed and immunostained for flag to visualize ALK and CLIMP63 to detect the ER. **(B)** Measurement of fluorescence intensity of HepG2 cells immunostained for endogenous LTK to demonstrate antibody specificity. Asterisk indicates statistically significant difference at $P < 0.05$ (*t* test). **(C)** Immunofluorescence against endogenous LTK and the ERES marker Sec31 in HepG2 cells. Region in white box is magnified, and the positions of colocalization events between LTK and Sec31 are highlighted by arrows. Scale bars in this figure are 15 µm.

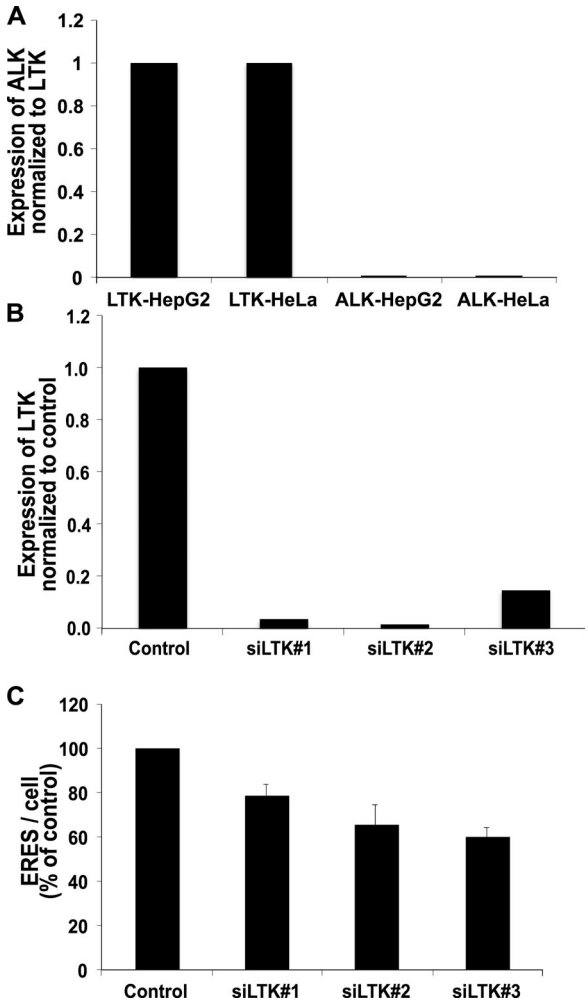


Figure S2. **Expression of ALK and LTK and effect of LTK depletion of ERESs. (A)** Expression of LTK and ALK mRNAs in HepG2 and HeLa cells assessed by qPCR. **(B)** Expression of LTK mRNA 72 h after knockdown with three different siRNAs targeting LTK from two independent experiments. **(C)** HeLa cells were transfected with the indicated siRNA and fixed after 72 h followed by immunofluorescence staining of Sec31 to label ERESs. Error bars represent SD.

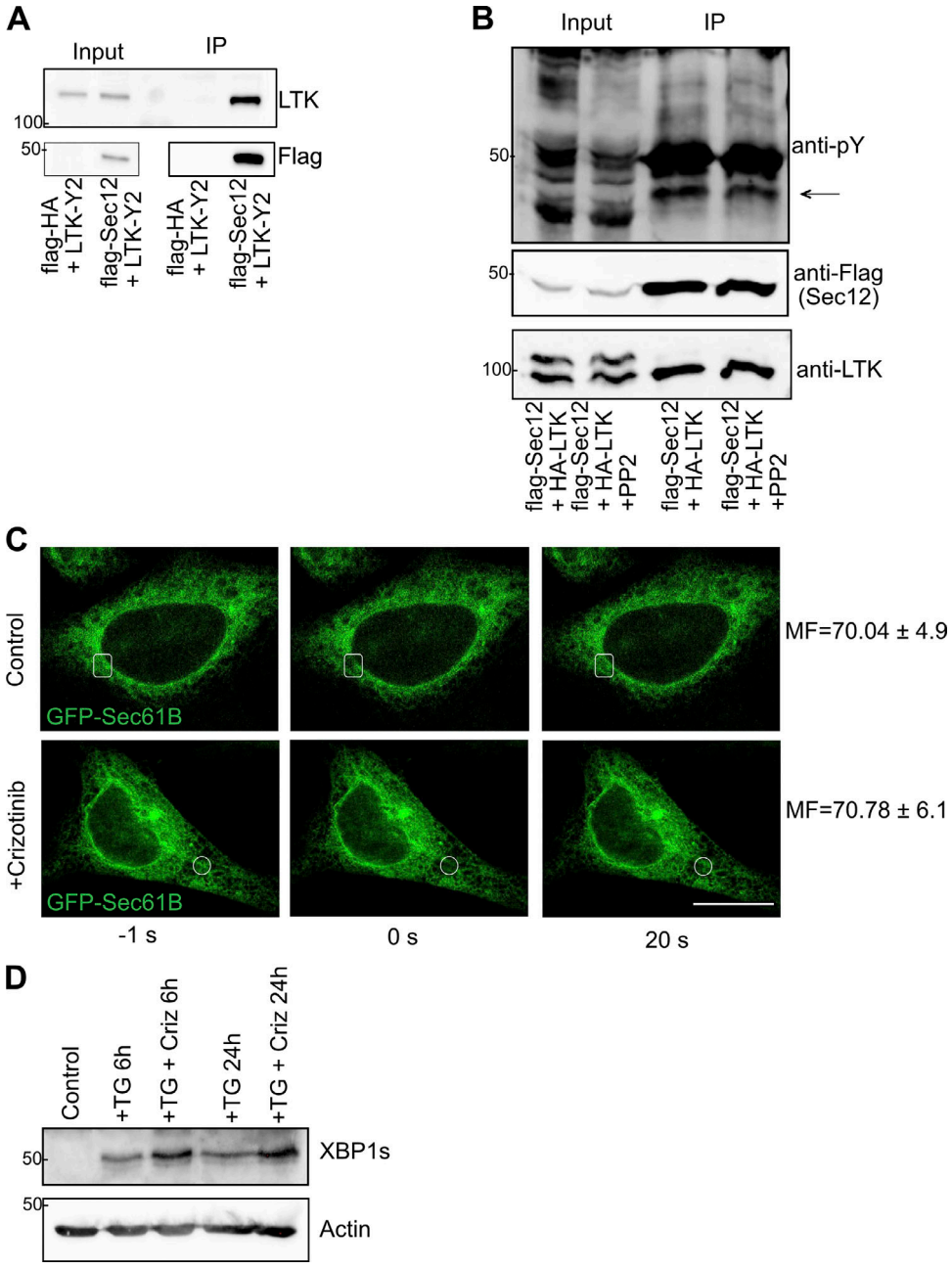


Figure S3. **Interaction of LTK with Sec12 as well as effect of LTK inhibition on ER homeostasis. (A)** HeLa cells expressing Y2-tagged LTK together with an empty flag vector or flag-tagged Sec12. Cells were lysed and subjected to anti-flag immunoprecipitation followed by immunoblotting as indicated. **(B)** HeLa cells expressing flag-tagged Sec12 and HA-tagged LTK were treated with solvent or with PP2 for 30 min before lysis and immunoprecipitation against flag. Immunoblotting was performed as indicated. Arrow indicates the position of phosphorylated Sec12 in the anti-phosphotyrosine blot. **(C)** FRAP microscopy of HeLa cells expressing the ER marker GFP-Sec61A. Cells were treated with solvent or with 1 µM crizotinib before the experiment. Bleaching was performed in the boxed region. MF, calculation of the mobile fractions from three independent experiments with at least six cells per experiment. Scale bar, 10 µm. **(D)** HeLa were treated with thapsigargin (1 µM) for the indicated time points, with or without 1 µM crizotinib. Cells were lysed and subjected to immunoblotting against spliced XBP1 (XBP1s) and β-actin to ensure equal loading. Control, cells treated with solvent.

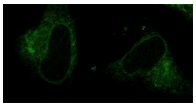
Table S1 is provided online as an Excel document and shows the results of the MS experiment of the LTK interactome.

Table S2. **List of antibodies used in this study**

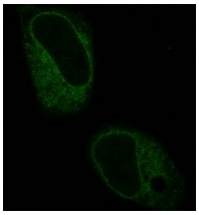
Antigen	Catalog no./source
FLAG-M2	F1804/Sigma-Aldrich
Climp-63	Rabbit/own
LTK	ap7658a/Abgent
Sec31	612351/BD Biosciences
FLAG-M2 HRP	A-8592/Sigma-Aldrich
P-tyrosine	Mouse/own
Giantin	ab80864/Abcam
Phospho-LTK	D59G10/Cell Signaling Technology
ERGIC-53	Rabbit/own
GFP (YFP2)	13026100/Roche
YFP1	ab32146/Abcam
GST	sc-138/Santa Cruz Biotechnology
Alexa Fluor 568 anti-mouse	A11004/Invitrogen
Alexa Fluor 568 anti-rabbit	A11011/Invitrogen
Alexa Fluor 488 anti-mouse	A11001/Invitrogen
Alexa Fluor 488 anti-rabbit	A11008/Invitrogen
Alexa Fluor 647 anti-mouse	A21235/Invitrogen
HRP rabbit	111035144/Jackson ImmunoResearch
HRP mouse	115035003/Jackson ImmunoResearch

Table S3. **List of PCR primers used in this study**

Gene	Sequence or catalog no.
ERGIC-53_C466Afw	5′-GTGGTAGTTCTGGGGCTTTCGGCTTTTCATTTGATGGCATATT-3′
ERGIC-53_C466Arev	5′-AATATGCCATCAAATGAAAAGCCGAAAGCCCCAGAACTACCAC-3′
ERGIC-53_C475Afw	5′-GAAGTGGACCGTAGACAAAGCTGATGGAAATGGTGGTAGT-3′
ERGIC-53_C475Arev	5′-ACTACCACCATTTCCATCAGCTTTGTCTACGGTCCACTTC-3′
SEC12Y10Ffw	5′-GAGCCCGGAACAGCTCTGGCGCCC-3′
SEC12Y10Frev	5′-GGGCGCCAGAGCTGTTCCGGGCTC-3′
SEC12Y177Ffw	5′-CTGGAGGAACAGATGGCTTCGTCCGTGTC-3′
SEC12Y177Frev	5′-GACACGGACGAAGCCATCTGTTCCTCCAG-3′
GST-SEC12fw	5′-ATTCTCGAGCATGGGCCGGCGCCGGGCG-3′
GST-SEC12rev	5′-AATTGCGGCCGCTCATTCATGGGACCCAAGGAG-3′
GAPDHfw	5′-ACAGTTGCCATGTAGACC-3′
GAPDHrev	5′-TTTTTGGTTGAGCACAGG-3′
LTK	QT00219877/Qiagen
ALK	QT00028847/Qiagen



Video 1. **Live imaging of a RUSH experiment in control and crizotinib-treated cells.** HeLa cells stably expressing the GFP-RUSH-Man-II construct (Str-KDEL-Man-II-EGFP). Live imaging was started immediately after addition of biotin. An image was acquired every 30 s, and cells were imaged for 20 min.



Video 2. **Live imaging of a RUSH experiment in control and crizotinib-treated cells.** HeLa cells stably expressing the GFP-RUSH-Man-II construct (Str-KDEL-Man-II-EGFP) were treated with 1 µM crizotinib for 30 min prior to addition of biotin. Imaging was started immediately after biotin addition. An image was acquired every 30 s, and cells were imaged for 20 min.