Supplemental Data

Figure S1. Characterization of MKS3 expression.

A. HEK293 cells were transiently transfected with WT (SP-C^{WT}) or mutant SP-C (SP-$C^{\Delta exon4}$ or SP-C^{L188Q}). 24 h later total RNA was isolated for analysis by real-time PCR.

B. HEK293 cells were transiently transfected with MKS3-FLAG. 48 h later cells were harvested and sonicated in buffer containing 0.6 M NaCl, 0.2 M Na_2CO_3, 0.1% Triton X-100 (TX) or 1% TX. Soluble (S) and insoluble (P) fractions were recovered by centrifugation and analyzed by SDS-PAGE/Western blotting with FLAG antibody. "T" is cell lysate prior to centrifugation.

C. Cell lysates from HEK293 cells transiently expressing MKS3-FLAG were prepared as described above. The cell pellet was resuspended in buffer with 0.2 mg/ml proteinase K and/or triton-X100 as indicated in the figure. After 30 min, samples were analyzed by SDS-PAGE/Western blotting with FLAG antibody. *FLJ14624* is a gene that was upregulated in parallel with *MKS3* in microarray analyses (1) and encodes a predicted membrane protein with a lumenal NH_2-terminus.

D. The sequence encoding residues 776-924 of human MKS3 was cloned into pET-28 and expressed in bacteria. Recombinant MKS3 was purified by Ni-NTA chromatography and analyzed by SDS-PAGE/silver staining (right panel). Purified peptide was injected into guinea pigs to generate polyclonal antisera as previously described (2). Western blotting of HEK293 cell lysate with MKS3 antibody detected one major protein band, Mr~120k (left panel).

E. Localization of MKS3 to HEK 293 cells demonstrated by cryo immunogold labeling. HEK 293 cells were labeled with MKS3 Ab and 10 nm protein A gold. Most of the endogenous MKS3 was associated with synthetic and secretory compartments such as ER and vesicles. Sparse labeling was also localized to the plasma membrane and lysosomes. ER, endoplasmic reticulum; MT, mtitochondria; MVB, multivesicular bodies; LYSO, lysosome; V, vesicles.

Figure S2. MKS3 interacts with mutant SP-C and ERdj5. HEK293 cells stably expressing SP-C^{WT} (WT) or SP-$C^{\Delta exon4}$ (Δx4) were transiently transfected with MKS3-FLAG and HA-tagged ERdj5. 48 h later cell cells were treated with MG-132 for 4 h and crosslinked with DSP for 30 min; lysates were immunoprecipitated with FLAG antibody and analyzed by SDS-PAGE/Western blotting with HA, SP-C or Flag antibodies.

Figure S3. siRNA-mediated inhibition of MKS3. HEK293 cells were transfected with 75 nM MKS3 siRNA pool (Dharmacon) or control non-targeting siRNA pool. 48 h later total RNA was isolated for RT-PCR analysis of MKS3 mRNA; cell lysates were analyzed by SDS-PAGE/Western blotting with MKS3 and GAPDH antibodies.

Figure S4. The lumenal domain of MKS3 interacts with mutant SP-C and ER chaperones.

A. The sequence encoding the predicted lumenal domain of MKS3 (residues 1-501) was cloned into p3xFLAG and transfected into HEK293 cells. $MKS3^{1-501}$ was detected by immunofluorescence using FLAG antibody. Calreticulin (CRT) was used as a marker for ER.

B. HEK293 cells stably expressing S-C^{WT} (WT) or SP-$C^{\Delta exon4}$ (Δx4) were co-transfected with $MKS3^{1-501}$ and HA-tagged ERdj4 or ERdj5. 24 h later cells were treated with MG-132 for 4 hr and crosslinked with DSP for 30 min. Cell lysates were immunoprecipitated with FLAG antibody and analyzed by SDS-PAGE/Western blotting with HA, SP-C, and FLAG antibodies.

Figure S5. Analyses of MKS3 mutants.

A. HEK293 cells were transfected with constructs encoding FLAG-tagged full-length MKS3, the lumenal domain of MKS3 ($MKS3^{1-501}$), the *wpk* rat MKS3 mutant ($MKS3^{P394L}$) or a human MKS3 mutant ($MKS3^{Q376P}$). 24 h later cells were labeled with ^{35}(S)met/cys for 30 min after which labeling media was replaced with chase media. Cell lysates were immunoprecipitated with FLAG antibody and analyzed by SDS-PAGE/autoradiography.

B. MEFs were isolated from E13 $wpk^{+/+}$ and $wpk^{-/-}$ embryos. HEK293 and MEF cell lysates were analyzed by SDS-PAGE/Western blotting with MKS3 and actin antibodies.

C. MEFs from $wpk^{+/+}$ and $wpk^{-/-}$ embryos were analyzed by immunofluorescence using MKS3 antibody.

Figure S6. MKS3 is required for ERAD of mutant α1-antitrypsin.

A. $Wpk^{-/-}$ MEFs were transfected with α1-antitrypsin (AAT) or mutant AAT (PiZ) cDNA. 48 h later cell lysates were analyzed by SDS-PAGE/Western blotting with AAT and GAPDH antibodies.

B. HEK293 cells were transfected with AAT or PiZ and MKS3-FLAG. 48 h later cells were crosslinked with DSP. Cell lysates were immunoprecipitated with AAT antibody followed by SDS-PAGE/Western blotting with FLAG and ATT antibodies.

References

1. Dong, M., Bridges, J. P., Apsley, K., Xu, Y. and Weaver, T. E. (2008) *Mol Biol Cell* **19**, 2620-2630
2. Stahlman, M. T., Besnard, V., Wert, S. E., Weaver, T. E., Dingle, S., Xu, Y., von Zychlin, K., Olson, S. J. and Whitsett, J. A. (2007) *J. Histochem. Cytochem.* **55**, 71-83

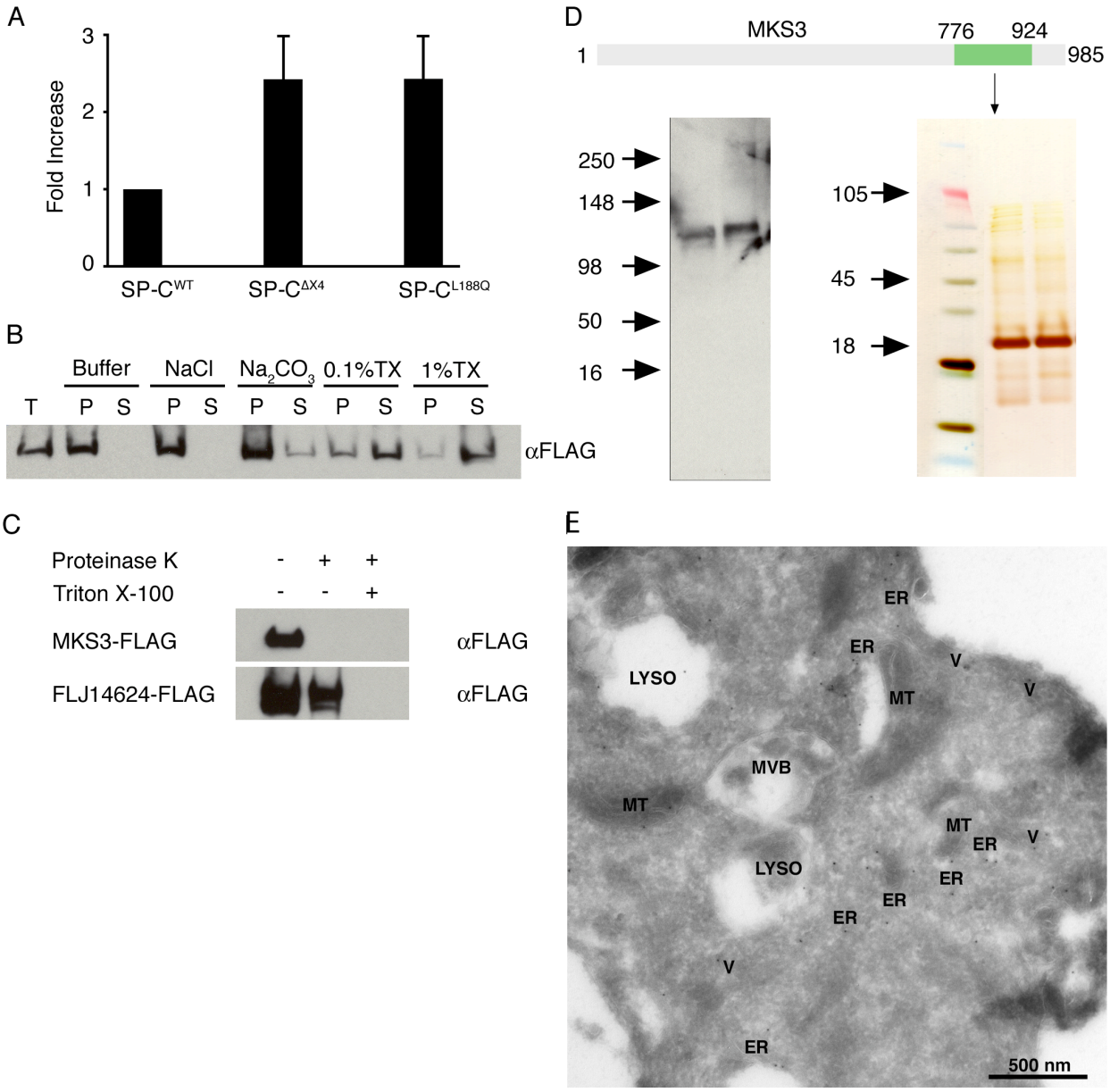
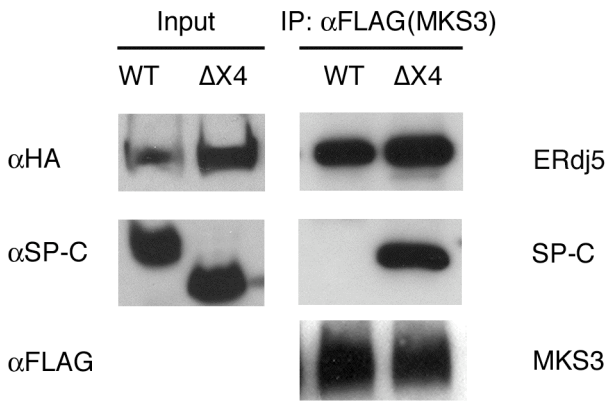

A
Fold Increase
3
2
1
0
SP-C^WT
SP-C^ΔX4
SP-C^L188Q
B
Buffer
NaCl
Na2CO3
0.1%TX
1%TX
T P S P S P S P S P S
αFLAG
C
Proteinase K - + +
Triton X-100 - - +
MKS3-FLAG
αFLAG
FLJ14624-FLAG
αFLAG
D
MKS3
776
924
1
985
250
148
98
50
16
105
45
18
E
ER
LYSO
V
MT
MVB
500 nm

FIGURE S1


Input
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ΔX4
WT
ΔX4
αHA
ERdj5
αSP-C
SP-C
αFLAG
MKS3

FIGURE S2

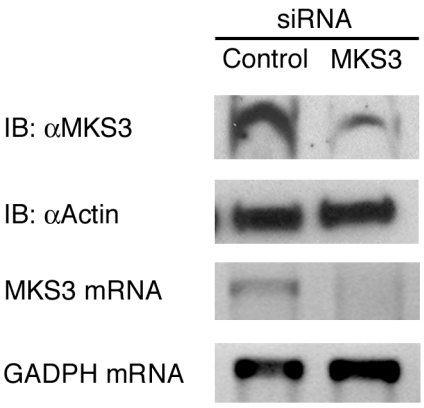

FIGURE S3

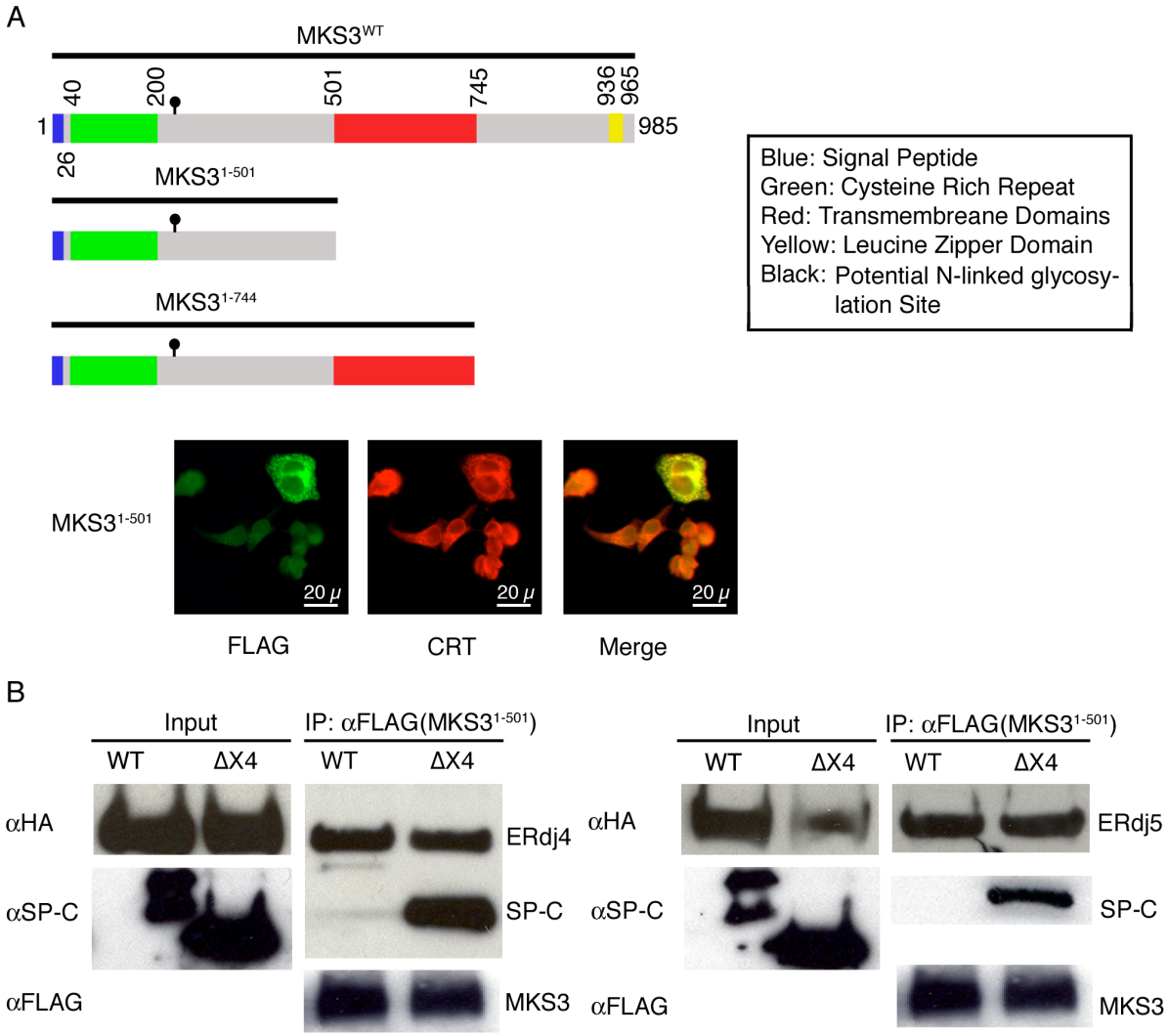
A
MKS3WT
40
200
501
745
936
965
1
985
26
MKS31-501
MKS31-744
Blue: Signal Peptide
Green: Cysteine Rich Repeat
Red: Transmembreane Domains
Yellow: Leucine Zipper Domain
Black: Potential N-linked glycosylation Site
MKS31-501
20 μ
20 μ
20 μ
FLAG
CRT
Merge
B
Input
IP: αFLAG(MKS31-501)
WT
ΔX4
WT
ΔX4
αHA
αSP-C
αFLAG
ERdj4
SP-C
MKS3
Input
IP: αFLAG(MKS31-501)
WT
ΔX4
WT
ΔX4
αHA
αSP-C
αFLAG
ERdj5
SP-C
MKS3

FIGURE S4

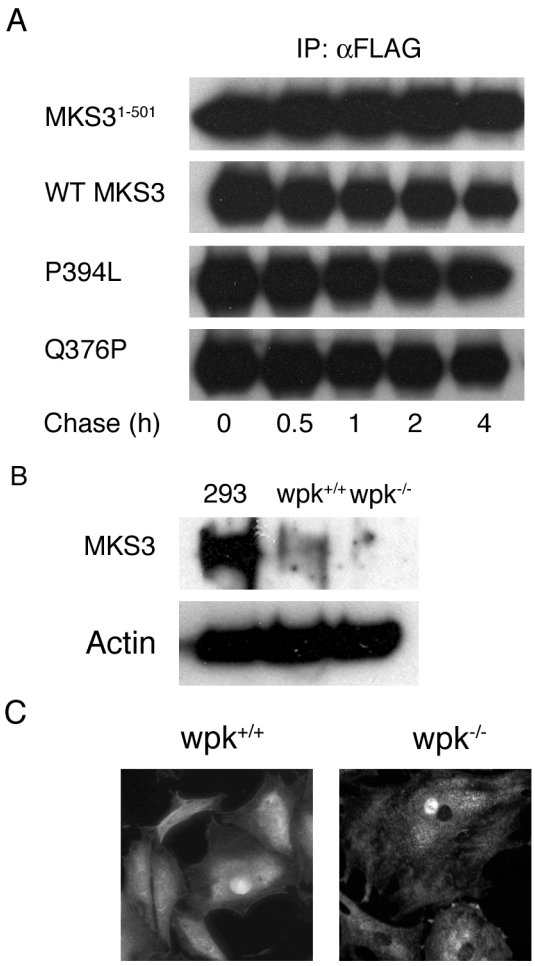
A
IP: αFLAG
MKS3 1-501
WT MKS3
P394L
Q376P
Chase (h) 0 0.5 1 2 4
B
293
wpk+/+
wpk-/-
MKS3
Actin
C
wpk+/+
wpk-/-

FIGURE S5

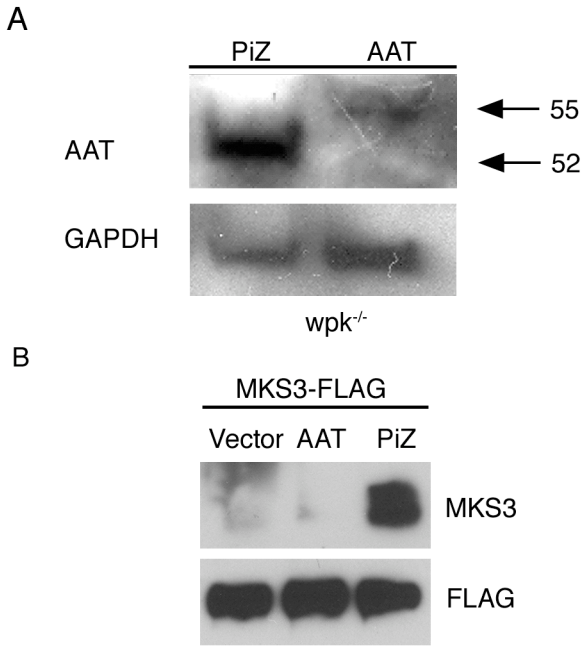
A
PiZ
AAT
55
52
AAT
GAPDH
wpk-/-
B
MKS3-FLAG
Vector
AAT
PiZ
MKS3
FLAG

FIGURE S6