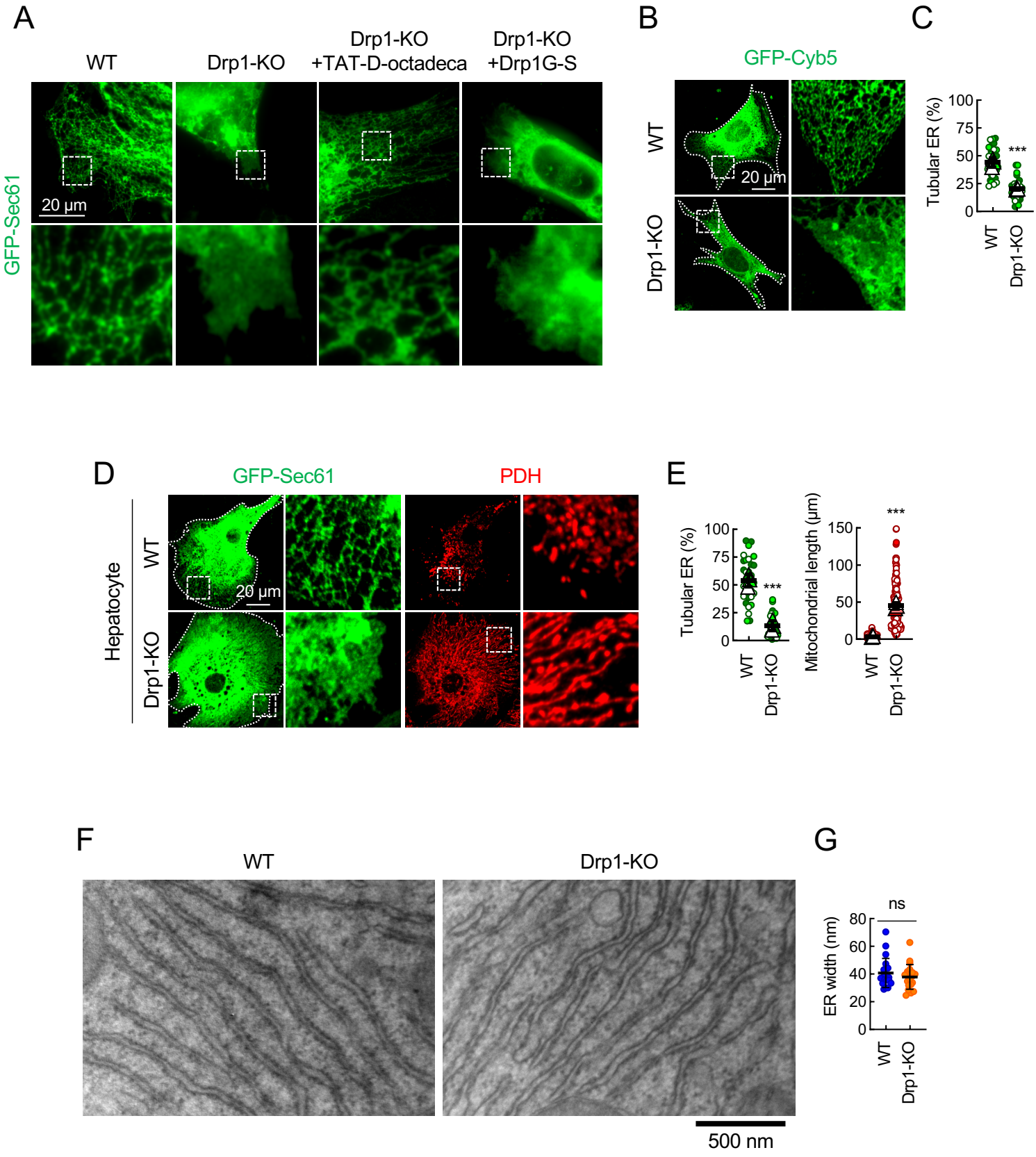


Adachi et al., Supplementary Figure S1

Supplementary Figure S1: Related to Figure 1

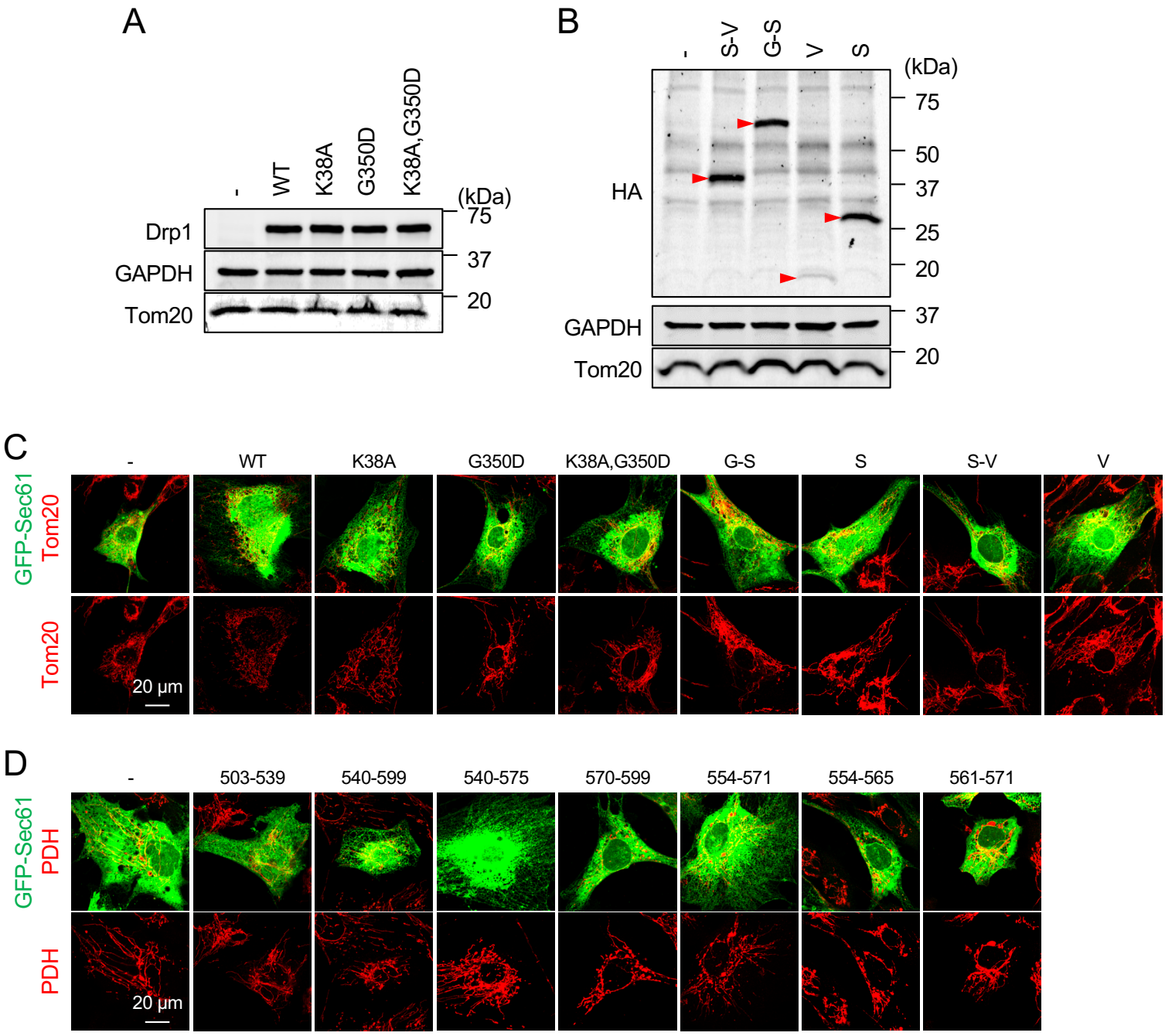
(A) WT MEFs, Drp1-KO MEFs, Drp1-KO MEFs treated with TAT-D-octadecapeptide, and Drp1-KO MEFs carrying Drp1G-S, all of which expressed GFP-Sec61β, were fixed and immunostained with anti-GFP antibodies. Structured illumination microscopy was used to view the ER.

(B and C) GFP-Cyb5 shows decreased levels of ER tubules in Drp1-KO MEFs. (B) WT and Drp1-KO MEFs were transfected with an ER marker (GFP-Cyb5) and subjected to immunofluorescence microscopy with anti-GFP antibodies. (C) Quantification of ER morphology. Bars are average ± SD (n = 3 experiments).

(D and E) Levels of ER tubules are decreased in Drp1-KO mouse primary hepatocytes. (D) WT and Drp1-KO hepatocytes were transfected with GFP-Sec61β and subjected to immunofluorescence microscopy with antibodies to GFP and PDH. (E) Quantification of the morphology of ER (green, n= 3) and mitochondria (red, n = 3).

(F and G) Electron microscopy of ER sheets in WT and Drp1-KO hepatocyte. (G) Quantification of the luminal width of ER sheets. Bars are the average ± SD (n = 20).

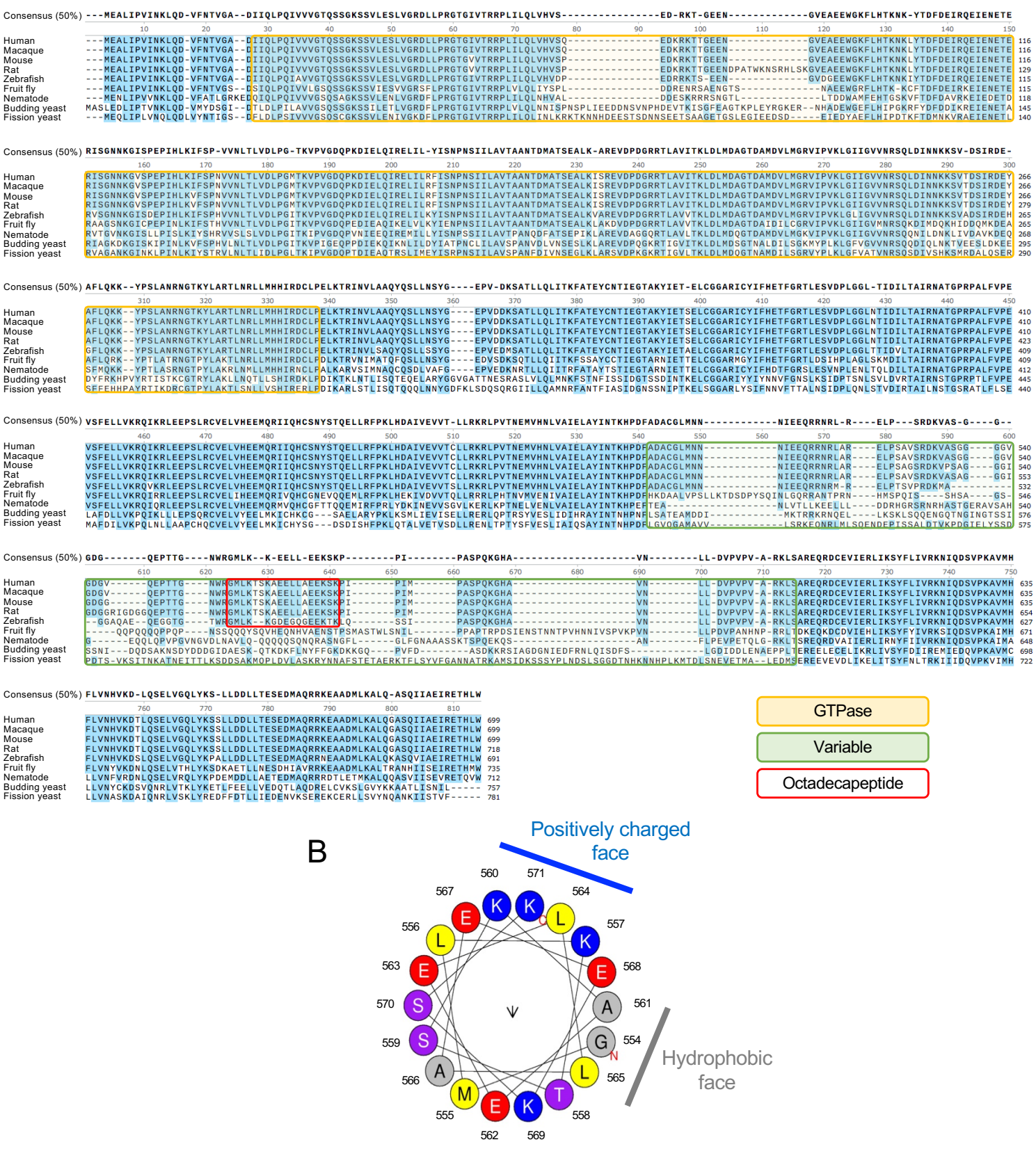
Statistical analysis was performed using Student's *t*-test (C, E and G): ***p<0.001.



Supplementary Figure S2: Related to Figure 5.

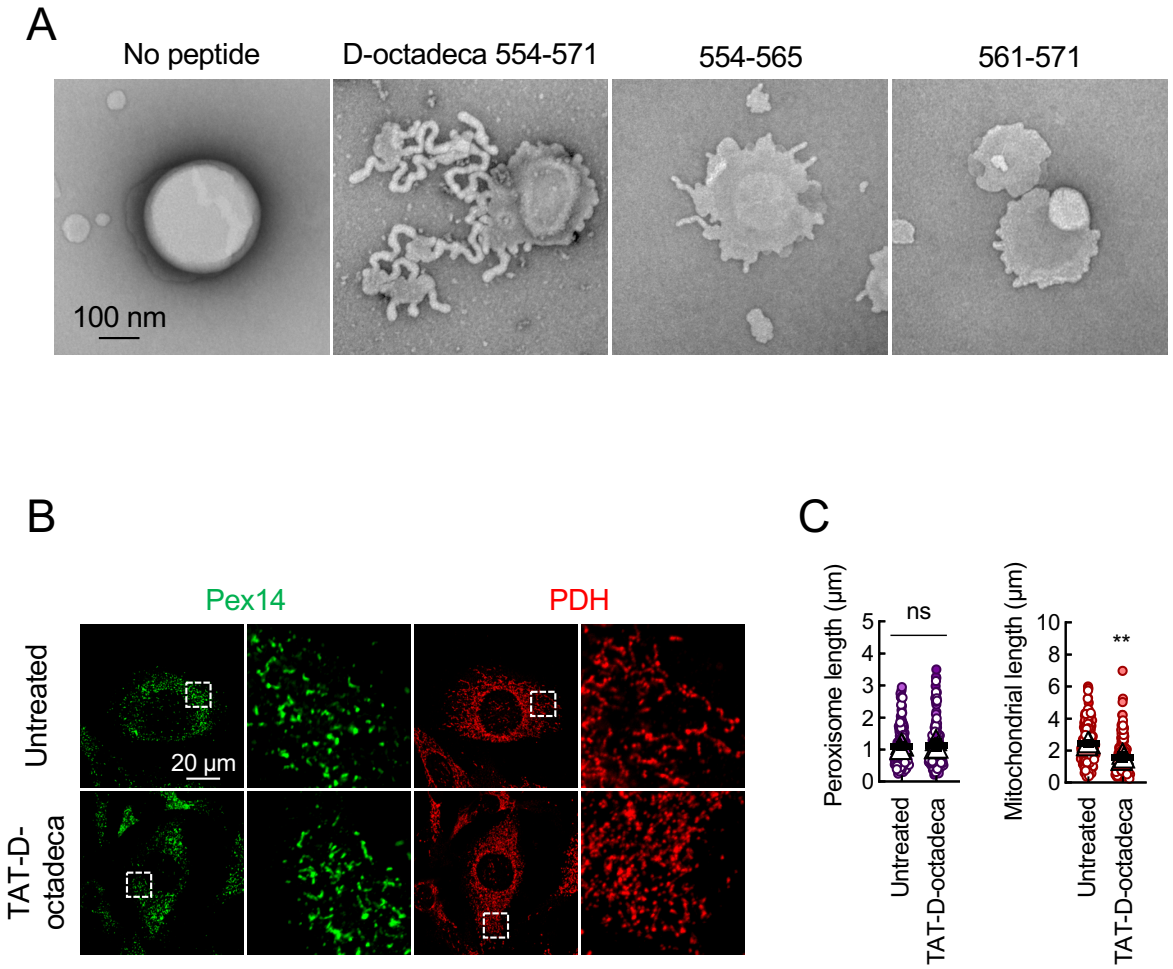
(A and B) Expression levels of WT and mutant Drp1 constructs. (A) Drp1-KO MEFs were transduced with the indicated Drp1 constructs and analyzed with Western blotting with antibodies to Drp1, GAPDH, and Tom20 in (A) and HA, GAPDH, and Tom20 in (B).

(C and D) Mitochondrial morphology of Drp1-KO MEFs expressing WT, mutant, and truncated Drp1. (C) Drp1-KO MEFs carrying GFP-Sec61β along with different Drp1 constructs were subjected to immunofluorescence confocal microscopy with antibodies to GFP and Tom20. The same set of MEFs was analyzed in this figure and Fig. 3B, in which only the GFP-Sec61β signal is presented. (D) Drp1-KO MEFs expressing the truncations of the variable domain were subjected to immunofluorescence confocal microscopy with antibodies to GFP and PDH. The same set of MEFs was analyzed in this figure and Fig. 3E, in which only the GFP-Sec61β signal is presented.



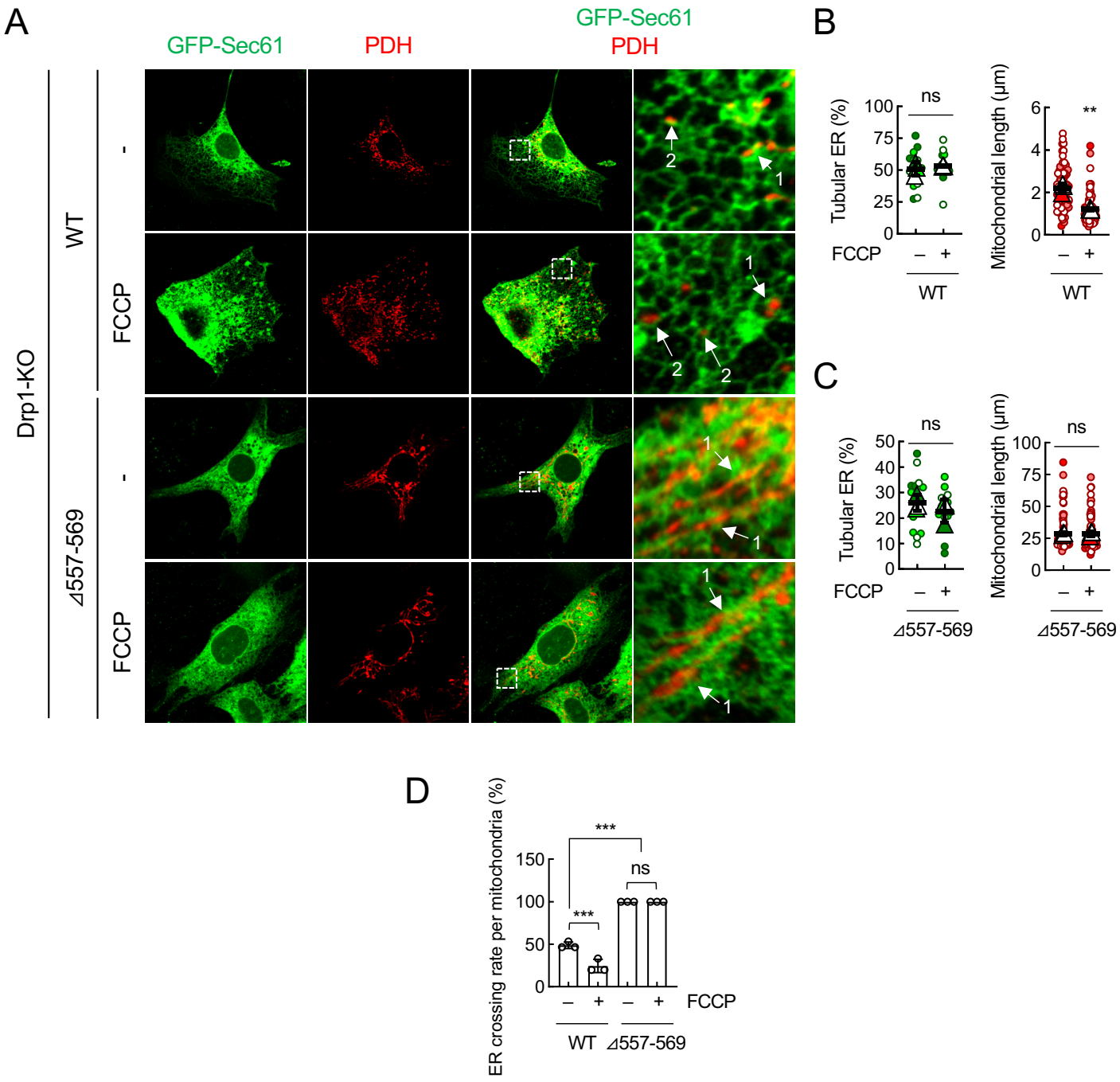
Supplementary Figure S3: Related to Figure 5

Analysis of amino acid sequences of Drp1 and D-octadecapeptide. (A) Drp1 proteins in the indicated species are aligned. Identical residues are highlighted in blue. The GTPase domain is boxed in yellow. The variable domain is boxed in green. The octadecapeptide sequence (554-571) is boxed in red. (B) The amino acid sequence of D-octadecapeptide was analyzed with HliQuest (http://heliquest.ipmc.cnrs.fr/cgi-bin/ComputParamsV2.py).

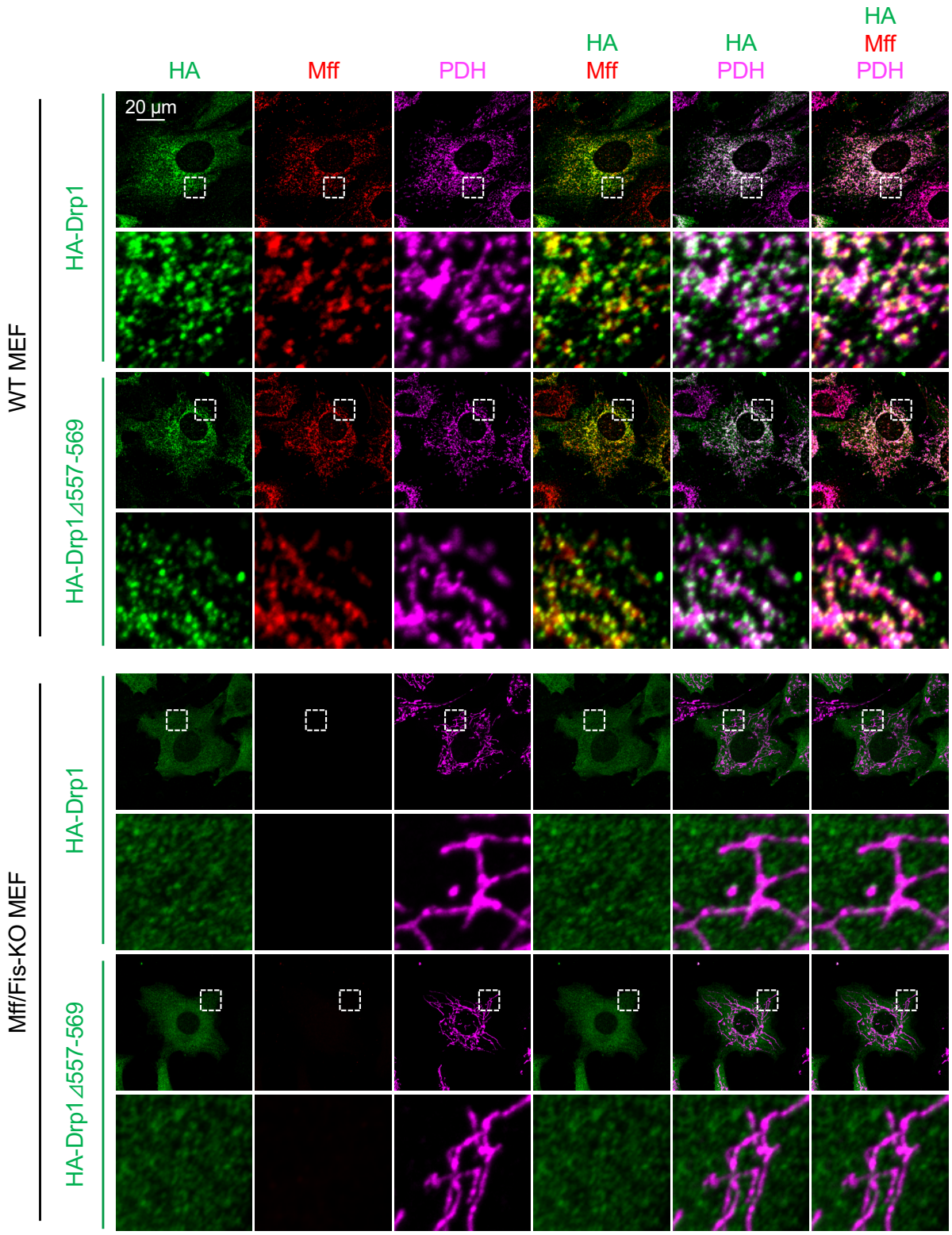


Supplementary Figure S4: Related to Figures 6 and 7.
(A) Liposome tubulation by truncated D-octadecapeptide. Liposomes that mimic the ER membrane carrying saturated PA were incubated with no peptide, full length D-octadecapeptide$_{554-571}$, or two truncated peptides (peptide$_{554-565}$ and peptide$_{561-571}$). Liposomes were analyzed by negative-stain EM.

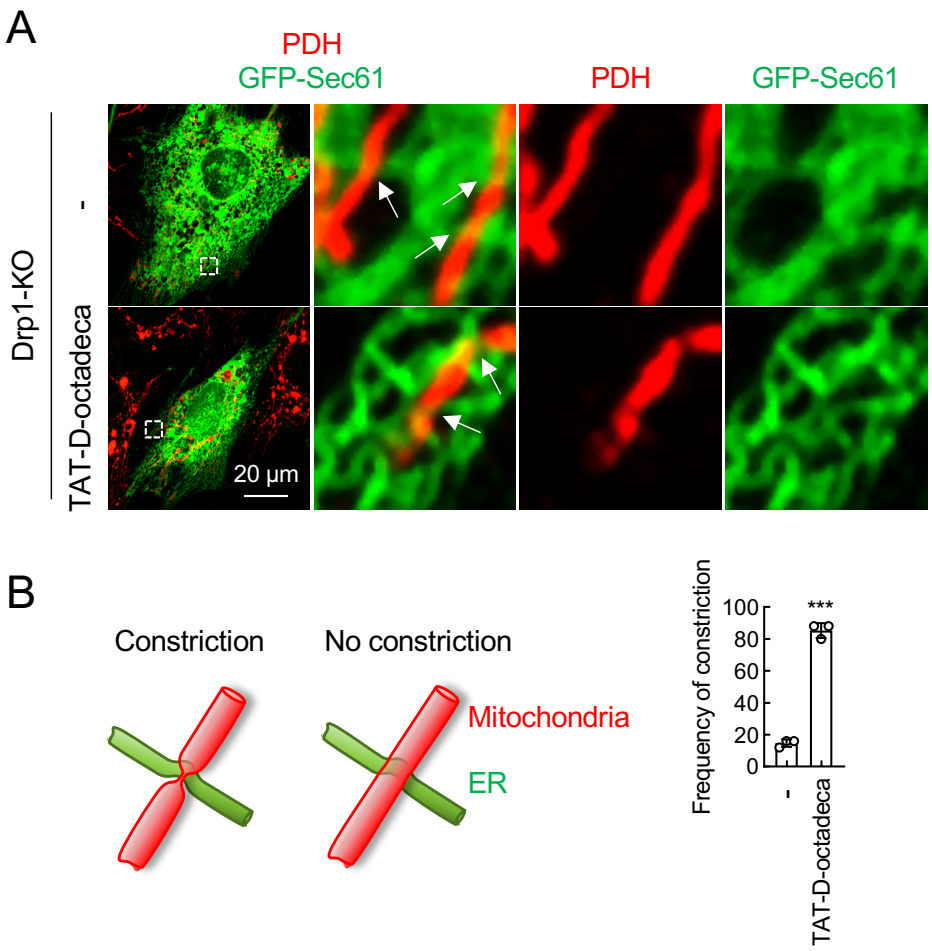
(B) WT MEFs were incubated with TAT-octadecapeptide and subjected to immunofluorescence microscopy using antibodies to Pex14 and PDH. Boxed areas are enlarged. (C) Quantification of the length of peroxisomes and mitochondria. Bars are the average ± SD (n = 3 experiments, each involving 50 peroxisomes and 50 mitochondria). Statistical analysis was performed using a student's t-test: **p<0.01.



Supplementary Figure S5, Related to Figure 7. (A) Drp1-KO MEFs carrying WT Drp1 or Drp1Δ557-569, along with GFP-Sec61β, were treated with 10 μM FCCP for 30 min. Cells were then subjected to immunofluorescence microscopy using antibodies to PDH and GFP. Arrows (1) indicate ER-mitochondria crossing, while arrows (2) indicate mitochondria located next to the ER. (B and C) Quantification of ER morphology and mitochondrial length. Bars are the average ± SD (n = 3). (D) The frequency of ER-mitochondria crossing when the ER and mitochondria are closely located. Bars are the average ± SD (n = 3 experiments). Statistical analysis was performed using a student's t-test in (B and D) and a one-way ANOVA with post-hoc Tukey test in (D): **p<0.001.



Supplementary Figure S6: Related to Figure 7.
WT and Mff/Fis1-KO MEFs were transduced with lentiviruses carrying HA-Drp1 or HA-Drp1Δ557-569 and subjected to immunofluorescence microscopy using antibodies to HA, Mff, and PDH. Boxed areas are enlarged.



Supplementary Figure S7, Related to Figure 7. (A) Drp1-KO MEFs were cultured in the presence or absence of TAT-D-octadecapeptide for 24 h. The ER and mitochondria were visualized by GFP-Sec61β and anti-PDH antibodies. Arrows indicate ER-mitochondria intersections (B) The frequency of mitochondrial constriction at mitochondria-ER intersections was determined. Bars are average ± SD (n = 3 experiments). In each experiment, 25 mitochondria were analyzed. Statistical analysis was performed using Student's *t*-test: ***p<0.001.