Supplementary data

Supplementary Figure 1. Peroxisomal and mitochondrial morphology in COS-7 cells.

(A-C) Peroxisomal (A) and mitochondrial (B) morphology in normal COS-7 cells (Con) processed for immunofluorescence microscopy. Peroxisomes in (A) were labeled with a polyclonal antibody to PMP70, a peroxisomal membrane protein, whereas mitochondria were stained with a monoclonal antibody to mangan superoxide dismutase (MnSOD) (Alexis Corporation, San Diego, CA, USA). Note the absence of colocalization in (C). Con, control.

(D-F) Expression of the peroxisomal membrane protein PMP70 does not alter peroxisomal morphology. COS-7 cells were transfected with a PMP70-Myc construct (kindly provided by S. Gould, Johns Hopkins University, Baltimore, MD, USA) and processed for immunofluorescence using antibodies to the Myc-epitope tag of PMP70 (D) and to peroxisomal catalase (E) (The Binding Site, Birmingham, UK). Note that peroxisomal morphology in COS-7 cells is not altered by overexpression of PMP70-Myc. Species-specific secondary antibodies conjugated to Alexa 488 (A, E) (Molecular Probes) and TRITC (B, D) were used. Bars, 10 µm.

Supplementary Figure 2. GFP-tagged and Myc-tagged hFis1 protein is targeted to peroxisomes.

COS-7 cells were transfected with either GFP-hFis1 (A-F) or Myc-hFis1 (G-O) and processed for immunofluorescence microscopy using antibodies to PMP70 (B, E, H, K, N) and the Myc epitope tag (G, J, M). Species-specific secondary antibodies conjugated to Alexa 488 (G, J, M) and Alexa 633 (B, E, H, K, N) (Molecular Probes) were used. Confocal images were acquired on a Leica TCS SP2 confocal microscope (Leica Microsystems, Heidelberg, Germany) using a ×63 water planapochromat objective. Digital images were optimized for contrast and brightness using Adobe Photoshop software. (D-F, J-L) Higher magnification

view of boxed region in (A, G). Note the slightly elongated peroxisomes in (J-L) (arrows). (M-O) Higher magnification view of another cell showing small, punctiform peroxisomes positive for Myc-hFis1. Bars, 10 µm.

Supplementary Figure 3. N-terminally truncated versions of hFis1 are targeted to peroxisomes without changing their morphology.

COS-7 cells were transfected with either hFis-YFP-TM/C (A-F) or Myc-hFis1(92-152) (G-L) and processed for immunofluorescence microscopy using antibodies to PMP70 (B, E, H, K) and the Myc epitope tag (G, J). Species-specific secondary antibodies conjugated to Alexa 488 (G, J) and Alexa 633 (B, E, H, K) (Molecular Probes) were used. Confocal images were acquired and processed as described in Supplementary Figure 2. (D-F, J-L) Higher magnification view of boxed region in (A, G). Note that the expression of hFis1-YFP-TM/C causes aggregation of mitochondria, whereas after expression of Myc-hFis1(92-152) mitochondrial and peroxisomal morphology is normal. Bars, 10 µm.

Supplementary Figure 4. The N-terminus of hFis1 is required for peroxisomal fission but dispensable for localization.

COS-7 cells were transfected with N-terminally truncated hFis1 constructs [Myc-hFis1(32-152) (E), Myc-hFis1(61-152) (A-D), Myc-hFis1(92-152) (E)], and immunostained with antibodies to the Myc tag of hFis1 (A, C, D) and to PMP70 (B, C, D). Cells expressing Myc-hFis1(61-152) and containing tubular mitochondria (A, C). Mitochondria in (A) are highly elongated and collapsed around the nucleus, whereas mitochondria in (C) show normal tubular morphology. Peroxisomal morphology and distribution is normal after expression of Myc-hFis1(61-152) (B-D). Note that in (A) contrast and brightness have been optimized for the visualization of mitochondrial morphology, and that therefore not all peroxisomes positive for Myc-hFis1(61-152) are visible. Arrows in (A, B) point to some regions of co-localization.

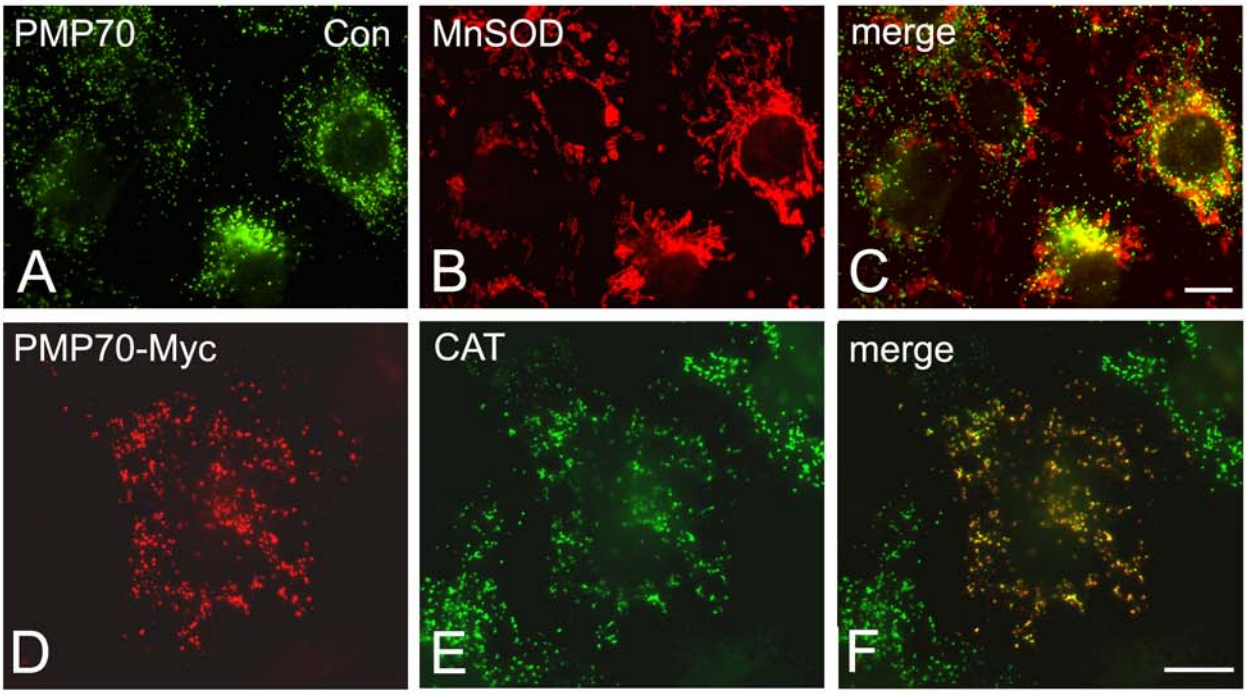
Co-staining (overlay) of Myc-hFis1(61-152) (green) and PMP70 (red) (C, D). Higher magnification image of boxed region in (C) is shown in (D). Note the peroxisomal localization of Myc-hFis1(61-152) (yellow). A quantitative analysis of peroxisome morphology after expression of the N-terminally truncated hFis1 constructs is shown in (E). Cells were categorized as cells with segmented (seg) or punctiform (punc) (% of total) peroxisomes (see Materials and methods). The data are from 4 independent experiments and are expressed as means ± S.D. Con, control (untransfected and vector only). Asterisks mark untransfected control cells. N, nucleus. Bars, 10 μm.

Supplementary Figure 5. Co-expression of GFP-hFis1 and Pex11pβ-Myc causes the formation of tubulo-reticular aggregates of peroxisomes.

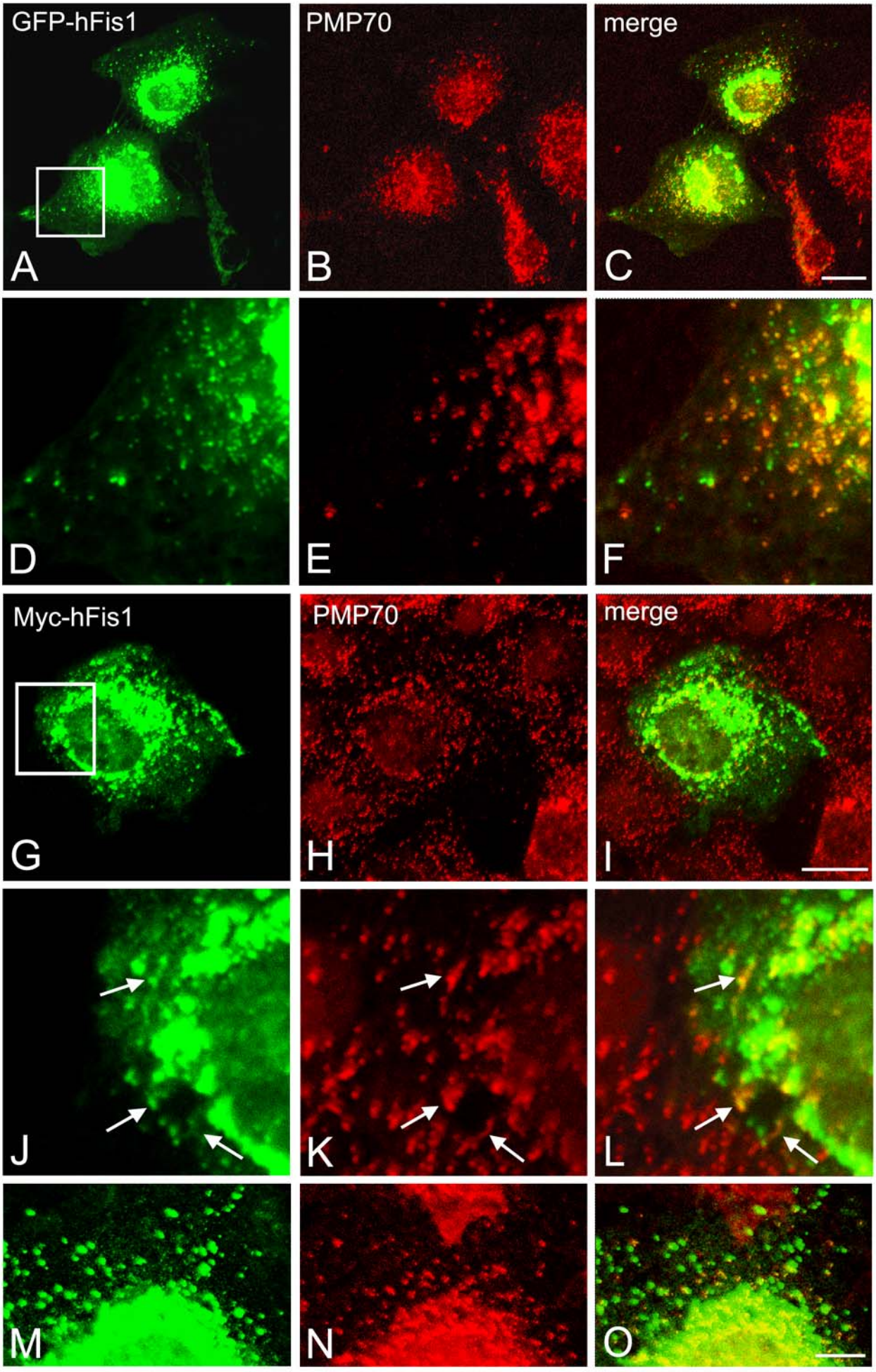
COS-7 cells were co-transfected with GFP-hFis and Pex11pβ-Myc and processed for immunofluorescence microscopy using antibodies to the Myc epitope tag (B, E, H, K, N). Species-specific secondary antibodies conjugated to Alexa 633 (B, E) or TRITC (H, K, N) were used. Confocal images (A-F) were acquired and processed as described in Supplementary Figure 2. (D-F) Higher magnification view of boxed region in (A) showing tubular peroxisomal aggregates. Note the co-localization of GFP-hFis1 and Pex11pβ-Myc (C, F). Deconvolution studies (G-O) were performed with a Soft Imaging System (Soft Imaging System GmbH, Münster, Germany) using an Olympus BX-61 microscope (Olympus Optical Co. GmbH, Hamburg, Germany) for optical sectioning. Deconvolved images were taken at 200 nm intervals with a 100× Plan-Neofluar objective with a 1.35 numerical aperture through focus. (G-I) Fluorescence localization of GFP-hFis1 (G) and Pex11pβ-Myc (H) in a co-transfected COS-7 cell. (J-L) Deconvolved image from a stack of images taken at 200 nm intervals of the boxed region in (G) showing tubular peroxisomal aggregates. (M-O) Deconvolved image from a stack of images taken from another cell showing a tubulo-reticular peroxisomal aggregate (arrow). Bars, 10 μm; 5 μm (M-O).

Supplementary Figure 6. Fis1 and Pex11pβ are not part of the same complex.

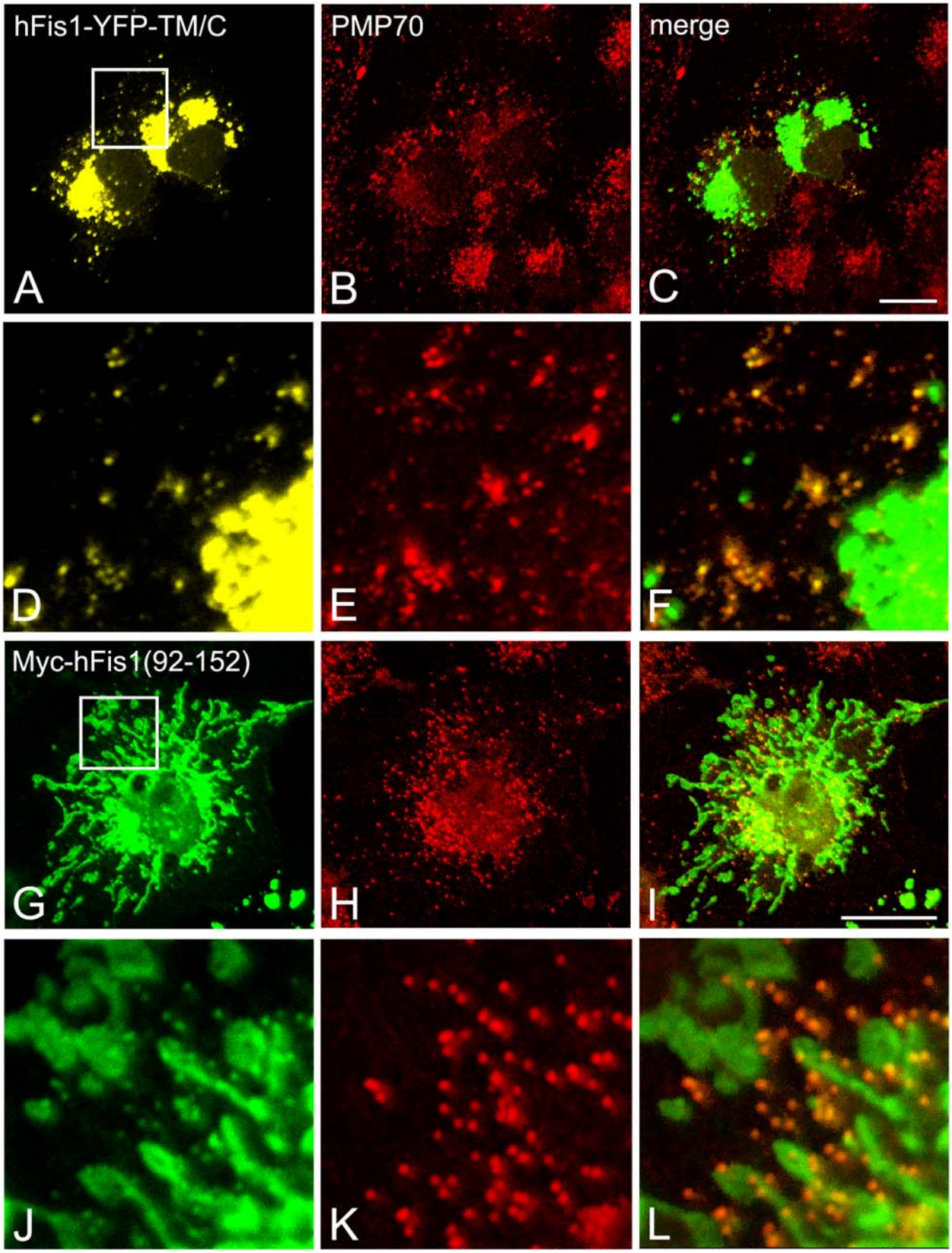
COS-7 cells were transfected with Pex11pβ-Myc (A), with Myc-hFis1 (B) or with buffer (IP Con), and were incubated for 48 hours. To stabilize potentially transient or weak interactions, whole cells were subjected to chemical cross-linking by incubation with the cleavable, cross-linker dithiobis(succinimidylpropionate) (DSP) (Pierce Chemical Co.) as described (Yoon *et al.*, 2003). For solubilization, conditions were used which have been shown to retain the ability of Pex11 proteins to interact with other proteins (Li and Gould, 2003). Cells were lysed by incubation with mixing in TBS buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1mM EDTA) containing 0.2% digitonin at 4°C for 1 hour. Lysates were cleared by centrifugation (100,000*g* for 30 minutes) (input). Immunoprecipitation (IP) was performed by adding 20 µl of a 25% slurry of anti-Myc monoclonal antibodies coupled to agarose beads (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and incubating with mixing at 4°C for 2 hours according to Li and Gould (2003). The beads were boiled in SDS-PAGE sample buffer containing 5% 2-mercaptoethanol to cleave the cross-linking. Samples were run on 12.5% acrylamide gels, and immunoprecipitated proteins were analyzed by immunoblotting with polyclonal anti-hFis1 (A, B), anti-DLP1 (A, B) and anti-Myc (**A**) antibodies. Fis1 and DLP1 were absent from immunoprecipitates obtained from Pex11pβ-Myc expressing cells, whereas immune complexes isolated from Myc-hFis1-expressing cells contained Fis1 and DLP1.



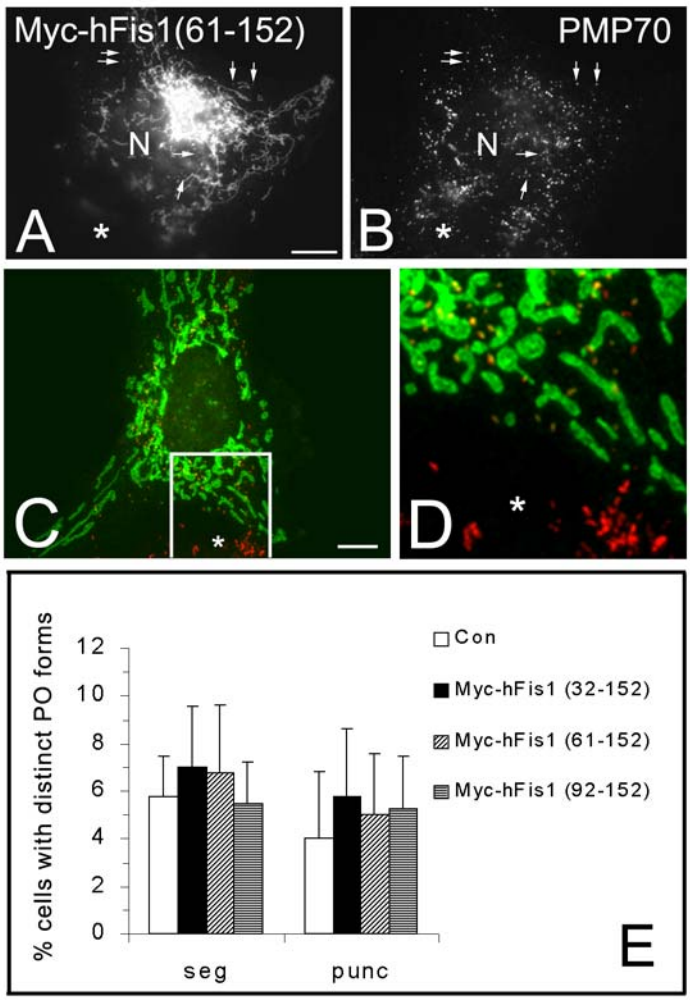
Supplementary Figure 1



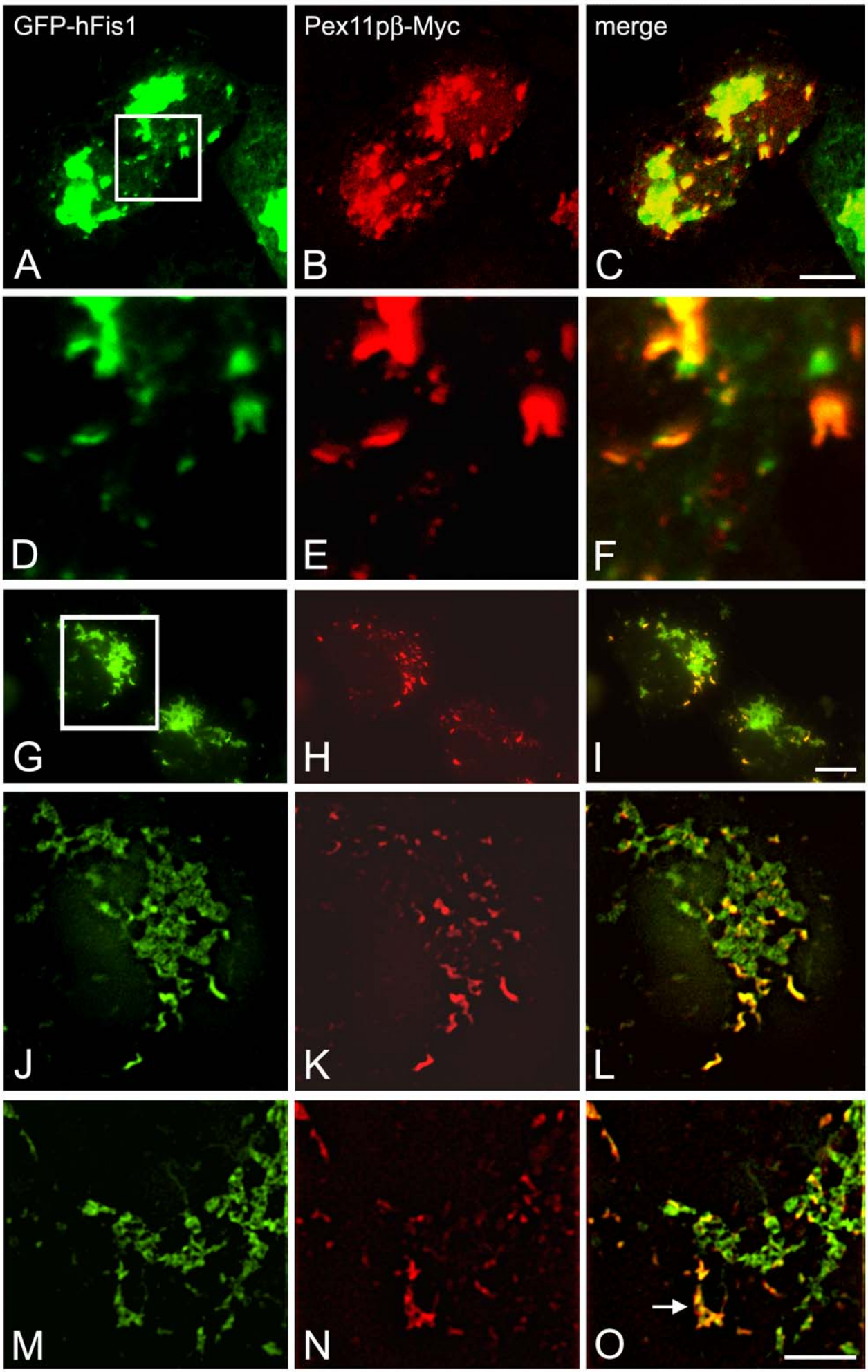
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5