Supplemental material JCB

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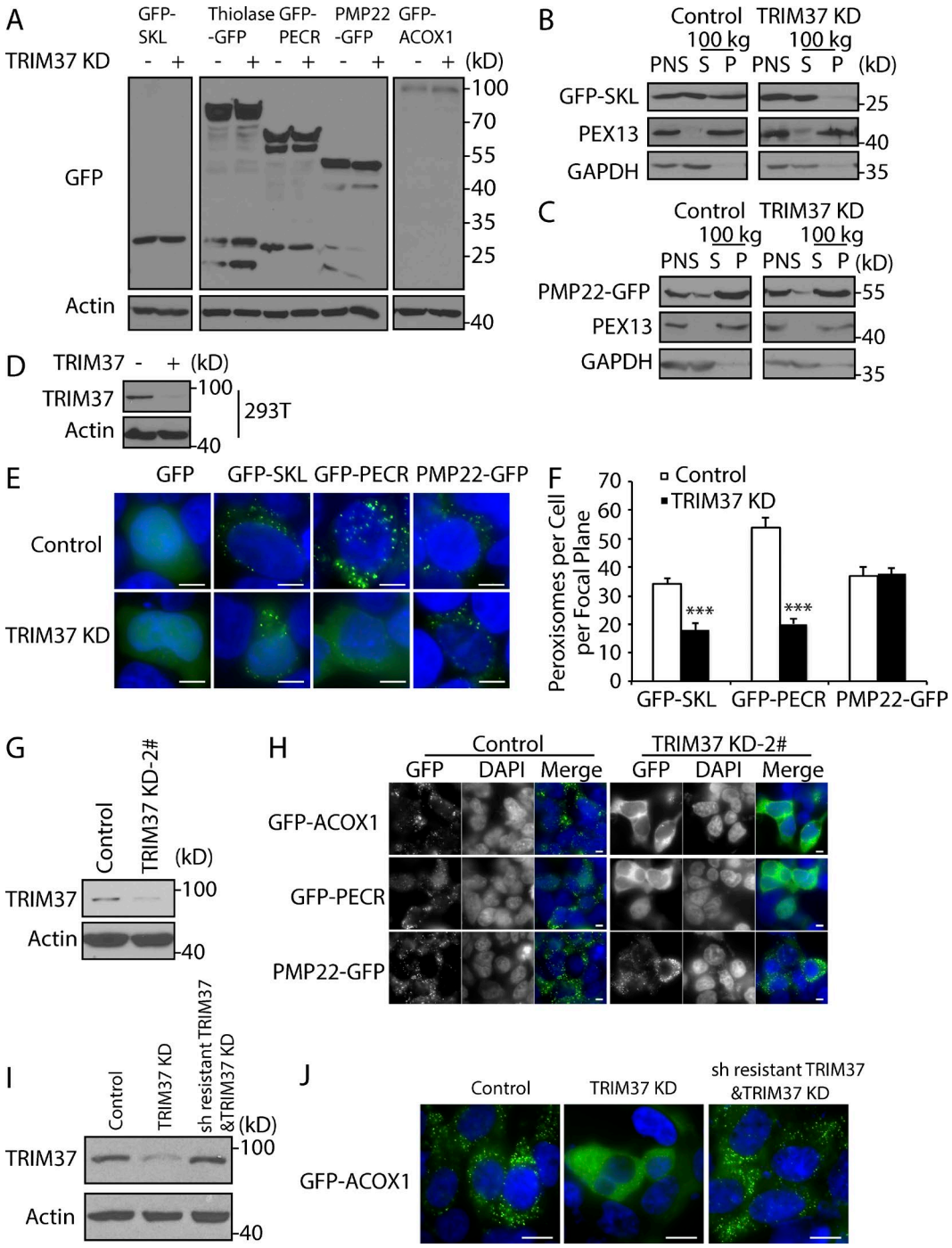

Figure S1. **Loss of TRIM37 impairs import of peroxisomal matrix proteins (related to Fig. 2).** (A) The indicated constructs were transfected into HepG2 control and TRIM37 KD cells as in Fig. 2 C. Total cell lysates were collected for Western blot detection of these fusion proteins using anti-GFP antibody. The amounts of the fusion proteins were equal between control and TRIM37 KD cells. (B and C) HepG2 control and TRIM37 KD cells were transfected with GFP-SKL or PMP22-GFP constructs. PNSs were fractionated into the cytosolic (S) and organelle (P) fractions by centrifugation at 100,000 *g*, followed by detection of GFP fusion proteins using anti-GFP antibody. (D) TRIM37 levels in HEK 293T control and TRIM37 KD cells. (E and F) HEK 293T control and TRIM37 KD cells were transfected with the indicated GFP constructs. The images were taken with the same exposure for each construct 24 h after transfection. Bars, 5 µm. The number of peroxisomes per cell per focal plane was calculated and presented as mean ± SEM in F. ***, $P < 0.001$ (Student's *t* test). The results are representative of three independent experiments. (G) TRIM37 KD efficiency in HepG2 TRIM37 KD-2# cells. (H) HepG2 control and TRIM37 KD-2# cells were transfected with GFP-ACOX1, GFP-PECR, or PMP22-GFP constructs. The images were taken 24 h after transfection. Bars, 5 µm. (I and J) The TRIM37 gene was silenced by shRNA in a HepG2 cell line expressing shRNA-resistant TRIM37 cDNA (short hairpin–resistant TRIM37 and TRIM37 KD) as described in Materials and methods. The GFP-ACOX1 construct was transfected into control, TRIM37 KD–, or short hairpin–resistant TRIM37 and TRIM37 KD HepG2 cells. The cell lysates were collected for the indicated antibody detection (I). The localization of GFP-ACOX1 was analyzed by fluorescence microscopy after 24 h (J). Bars, 10 µm.

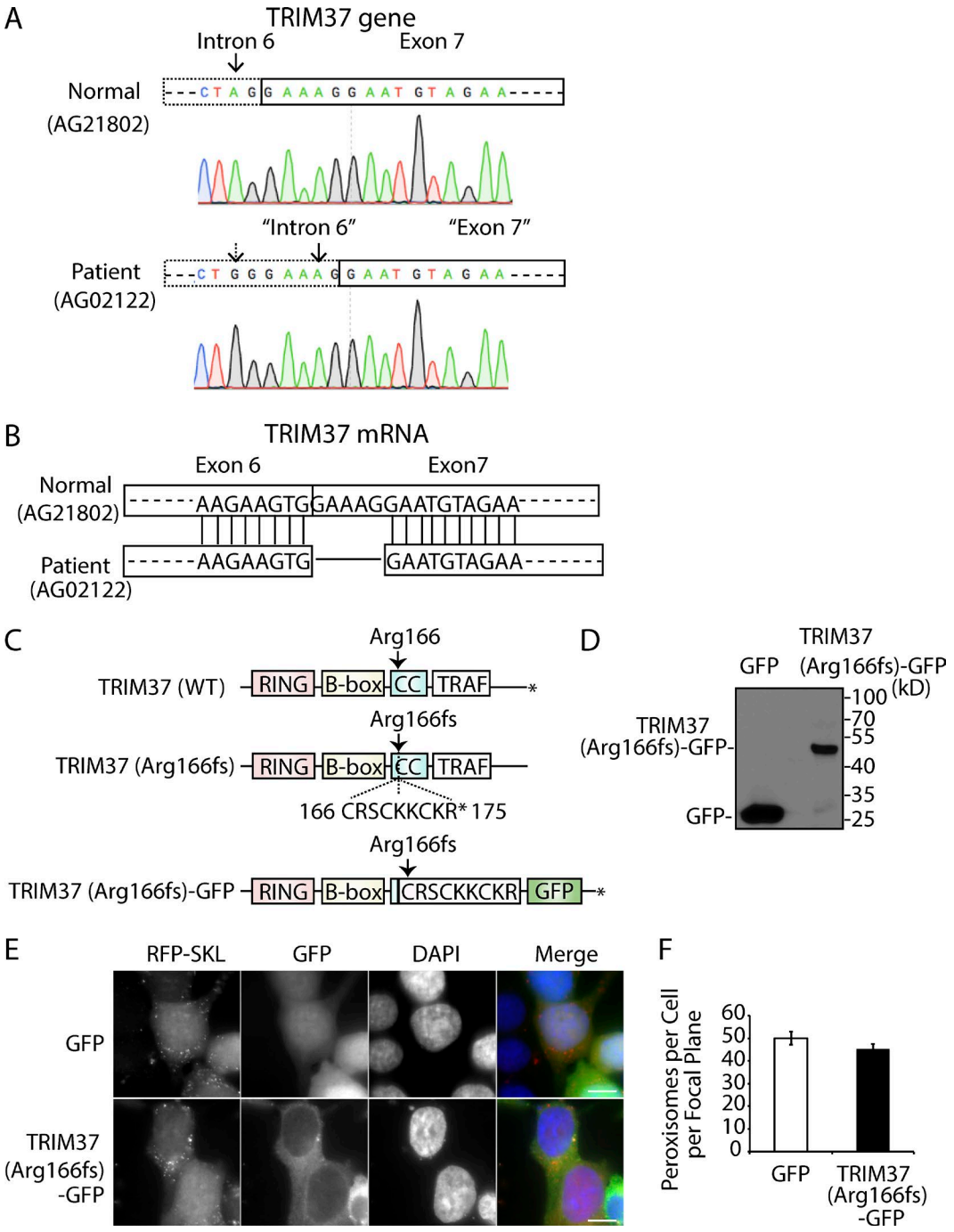

Figure S2. ***TRIM37* gene mutation results in defective import of peroxisomal matrix proteins (related to Fig. 3).** (A) Genomic DNA were extracted from normal (AG21802) and patient fibroblasts (AG02122) and used as the template for PCR amplification of the region surrounding the genomic mutation site (c.493-2A>G). The PCR products were sequenced to verify the gene mutation as indicated by the arrows. (B) RNA was extracted from normal and patient fibroblasts for cDNA synthesis. The region surrounding the mutation sites was amplified by PCR using the cDNA as a template, and the products were sequenced. The cDNA sequences in the region of the TRIM37 mutation between normal and patient cells were aligned. (C) Diagram of TRIM37 (WT), TRIM37 (Arg166fs) structures, and TRIM37 (Arg166fs)–GFP construct. (D) GFP or TRIM37 (Arg166fs)–GFP construct was transfected into HepG2. Cell lysates were collected after 24 h for detection of GFP or GFP fusion proteins using anti-GFP antibody. (E and F) HepG2 cells were transfected with constructs expressing RFP-SKL together with either GFP or TRIM37 (Arg166fs)–GFP. The images were taken after 24 h. The peroxisome numbers per cell and per focal plane are presented as mean ± SEM. Bars, 5 μm.

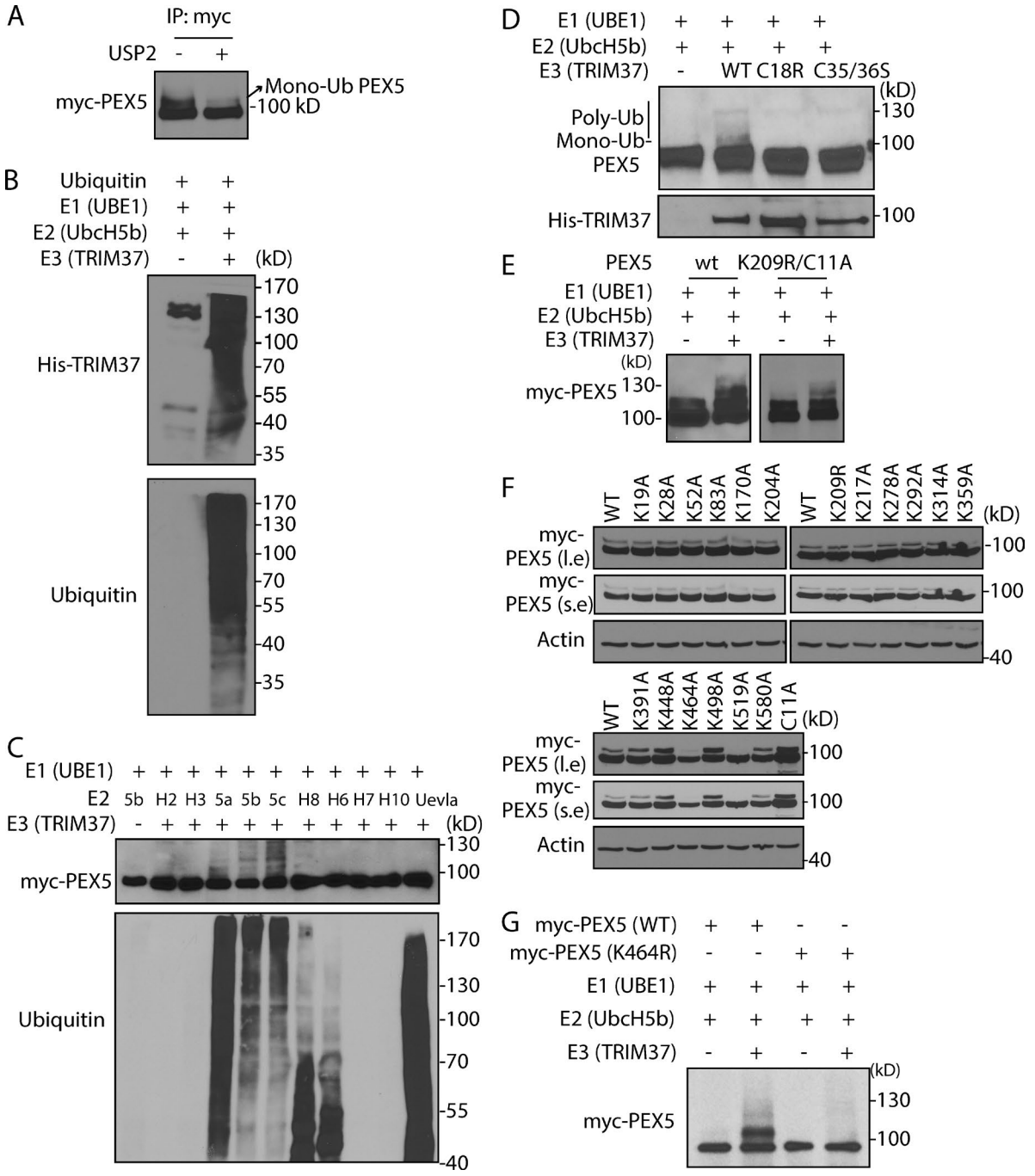

Figure S3. **TRIM37 ubiquitylates PEX5 at K464 (related to Fig. 4).** (A) Myc-PEX5 construct was transfected into HEK 293T cells, and myc-IP was performed 24 h later. Myc-PEX5 IP beads were treated with the deubiquitylating enzyme, 10 nM USP2, for 1 h or left untreated followed by detection with anti-myc antibody. (B) Self-ubiquitylation activity of TRIM37 by using UBE1, UbcH5b, and purified bacterial TRIM37 protein. Histidine (top) and ubiquitin (bottom) antibodies were used for protein detection. (C) In vitro ubiquitylation assay with UBE1, different E2s as indicated, and purified bacterial TRIM37 protein. PEX5 in the myc-IP of myc-PEX5–overexpressing HEK 293T cells was detected using anti-myc antibody (top), and ubiquitylated proteins (bottom) were detected using anti-ubiquitin antibody. H2, UbcH2; H3, UbcH3; 5a, UbcH5a; 5b, UbcH5b; 5c, UbcH5c; H6, UbcH6; H8, UbcH8; H7, UbcH7; H10, UbcH10. (D) In vitro ubiquitylation assay with UBE1, UbcH5b, purified bacterial TRIM37 (WT and C18R or C35/36S mutants), and histidine-PEX5 protein purified from *E. coli*. The amount of TRIM37 protein was determined by histidine antibody before the ubiquitylation assay, and PEX5 was detected with anti-PEX5 antibody. (E) In vitro ubiquitylation assay using UBE1, UbcH5b, and purified bacterial TRIM37 protein and myc-PEX5 (WT) or myc-PEX5 (K209R/C11A) immunoprecipitated from myc-PEX5–overexpressing HEK 293T cells. Myc-PEX5 was detected with anti-myc antibody. (F) Each single lysine in PEX5 was mutated to alanine (except K209R and C11A). The numbers indicate the position of the mutated lysines or cysteine. These PEX5 mutants were transfected into HEK 293T cells for detection of myc-PEX5 with anti-myc antibody. s.e, short exposure; l.e, long exposure. (G) In vitro ubiquitylation assay using myc-PEX5 (WT) or PEX5 (K464R) with components indicated. Immunoblot was with anti-myc antibody.

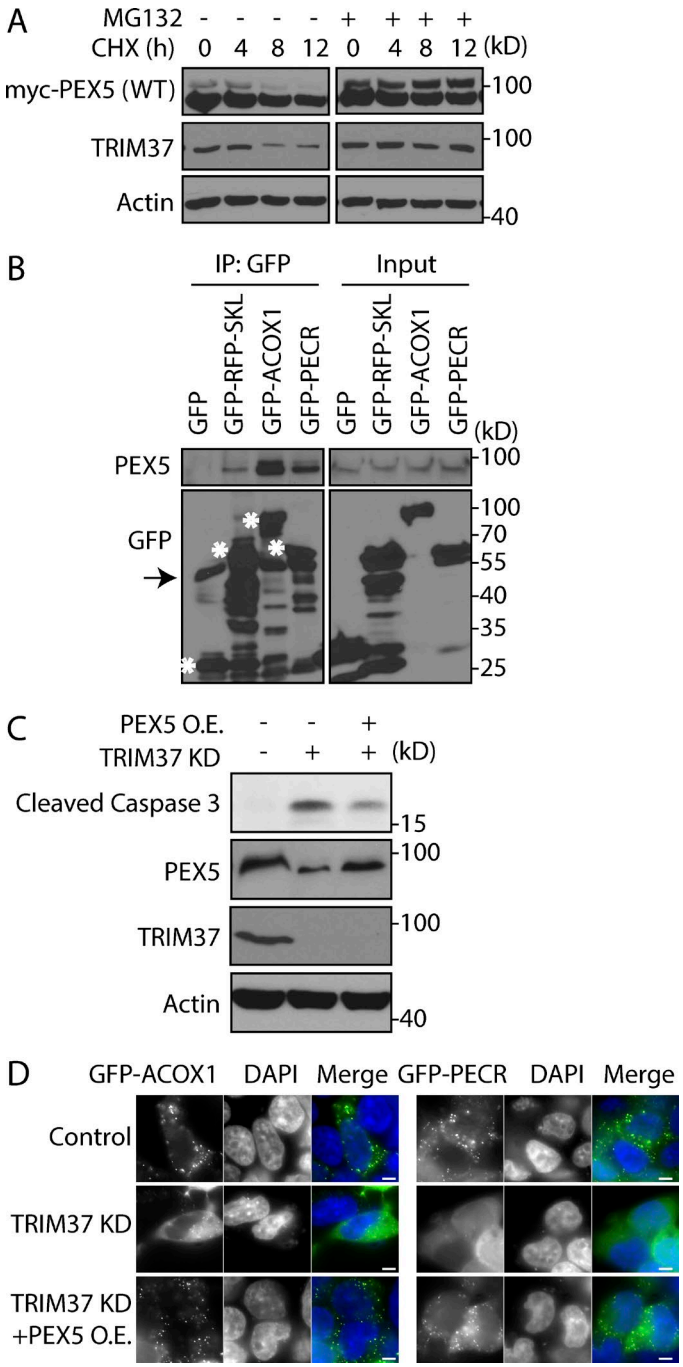

Figure S4. **Proteasomal degradation of PEX5 and TRIM37, PTS1 cargo interactions with PEX5, and rescue of PTS1 protein import by PEX5 overexpression in TRIM37 KD cells (related to Figs. 6, 8, and 9).** (A) Myc-PEX5 (WT) was transfected into HepG2 cells. 24 h later, cells were treated with 50 µg/ml CHX in the absence or presence of 10 µM MG132 for the indicated times. Myc-PEX5 and TRIM37 proteins were detected with anti-myc and anti-TRIM37 antibodies, respectively. (B) Interaction between PEX5 and several PTS cargoes. HepG2 cells were transfected with GFP, GFP-RFP-SKL, GFP-ACOX1, or GFP-PECR. Cell lysates were collected 24 h after transfection for GFP-IP. The asterisks in the left panel indicate the positions of immunoprecipitated GFP or GFP fusion proteins. The arrow indicates IgG heavy chain bands. PEX5 was detected with anti-PEX5 antibody. (C) TRIM37 was knocked down in HepG2 cells and PEX5-overexpressing HepG2 cells. The cell lysates were collected for detection of the indicated proteins with antibodies. (D) GFP-ACOX1 or GFP-PECR construct was transfected into the indicated cell lines, followed by microscopy analysis of the distributions of the expressed proteins. Bars: 5 µm.

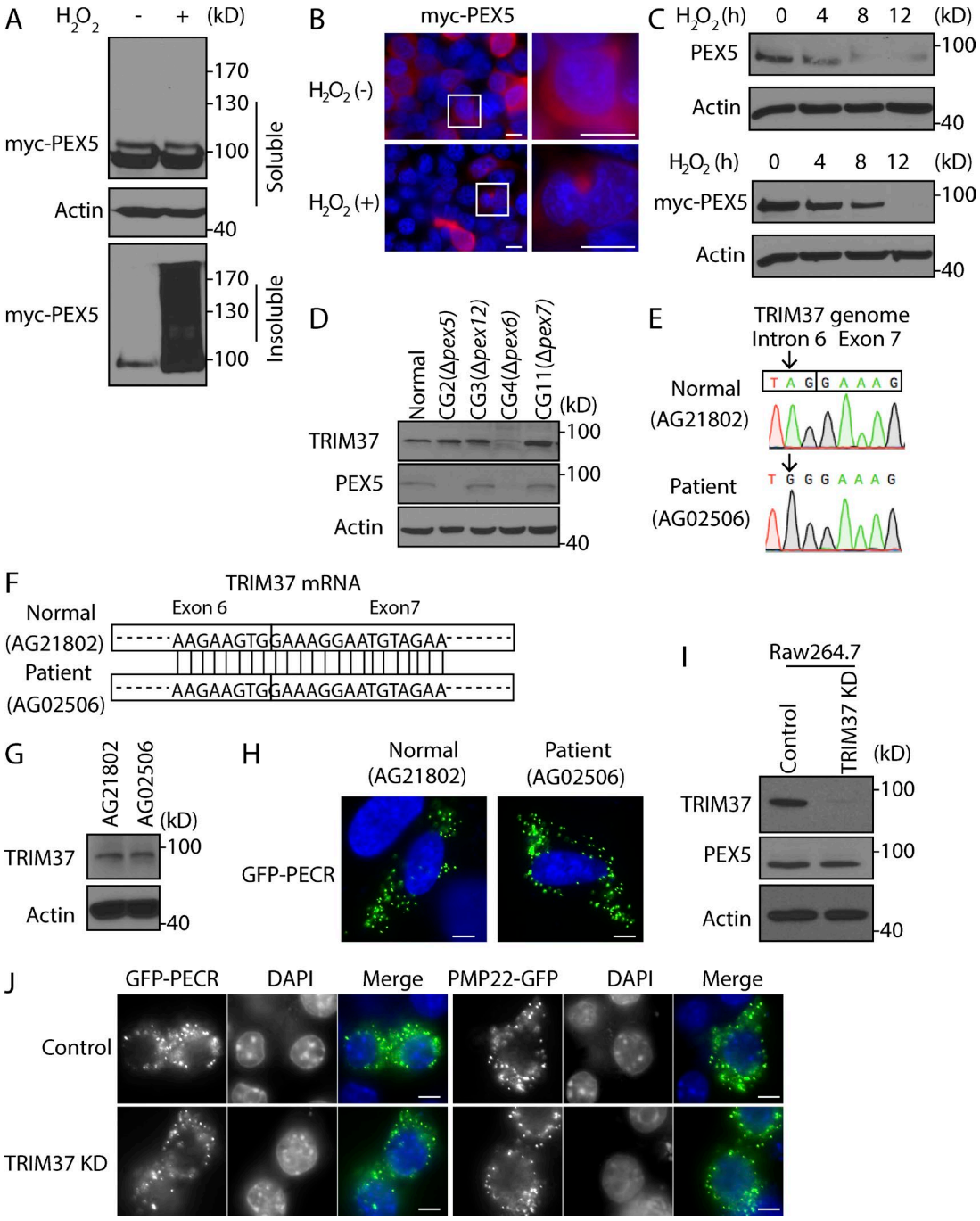

Figure S5. **Data for Discussion section.** (A–C) H_2O_2 induces PEX5 polyubiquitylation and degradation. (A) HEK 293T cells were transfected with myc-PEX5 and ubiquitin (WT). Cells were treated with 1 mM H_2O_2 for 4 h, a day after transfection. Cell lysates were collected in 1% CHAPS buffer and centrifuged at 12,000 rpm for 5 min. The supernatants and pellets were collected as soluble and insoluble fractions, respectively. Exogenous myc-PEX5 was detected with anti-myc antibody. (B) HEK 293T cells transfected with myc-PEX5 and ubiquitin (WT) were either treated with 1 mM H_2O_2 or not for 4 h, followed by immunostaining of myc-PEX5 protein using myc antibody. The panel on the right shows a higher magnification of the white boxes on the left. Bars: 10 µm. (C, top) HepG2 cells were treated with 1 mM H_2O_2 for the indicated times, and the endogenous PEX5 level was monitored using anti-PEX5 antibody. (Bottom) HepG2 cells were transfected with the construct expressing myc-PEX5. The cells were treated with 1 mM H_2O_2 for the indicated times 24 h after transfection. Exogenous myc-PEX5 was detected in cell lysates using anti-myc antibody. (D) TRIM37 and PEX5 protein levels in PBD fibroblasts. The indicated cell lysates were collected for Western blot detection of TRIM37 and PEX5 proteins by using anti-TRIM37 and anti-PEX5 antibodies, respectively. (E–H) Import of peroxisomal matrix protein is normal in patient AG02506. (E) Genomic DNAs were extracted from normal (AG21802) and patient fibroblasts (AG02506) and used as the template for PCR amplification of the region surrounding the genomic mutation site (c.493-2A>G). The PCR products were sequenced to verify the gene mutation as indicated by the arrows. (F) RNA was extracted from normal (AG21802) and patient fibroblasts (AG02506) for cDNA synthesis. PCR amplification of regions surrounding the mutation sites was performed using the cDNA as a template, and the products were sequenced. The cDNA sequences in the region of the mutation between normal and patient cells were aligned. (G) Protein lysates were extracted from normal (AG21802) and patient fibroblasts (AG02506) for Western blot detection of TRIM37. (H) GFP-PECR–expressing construct was transfected into normal (AG21802) and patient fibroblasts (AG02506) and imaged. Bars: 10 µm. (I and J) Import of PMP and PTS proteins is normal in Raw264.7 TRIM37 KD cells. (I) TRIM37 and PEX5 protein expression in control and TRIM37 KD Raw264.7 cells. (J) Constructs expressing either GFP-PECR or PMP22-GFP were transfected into Raw264.7 control or TRIM37 KD cells. The GFP fusion proteins were imaged after 24 h. Bars: 5 µm.