Hosoi et al., https://doi.org/10.1083/jcb.201605002

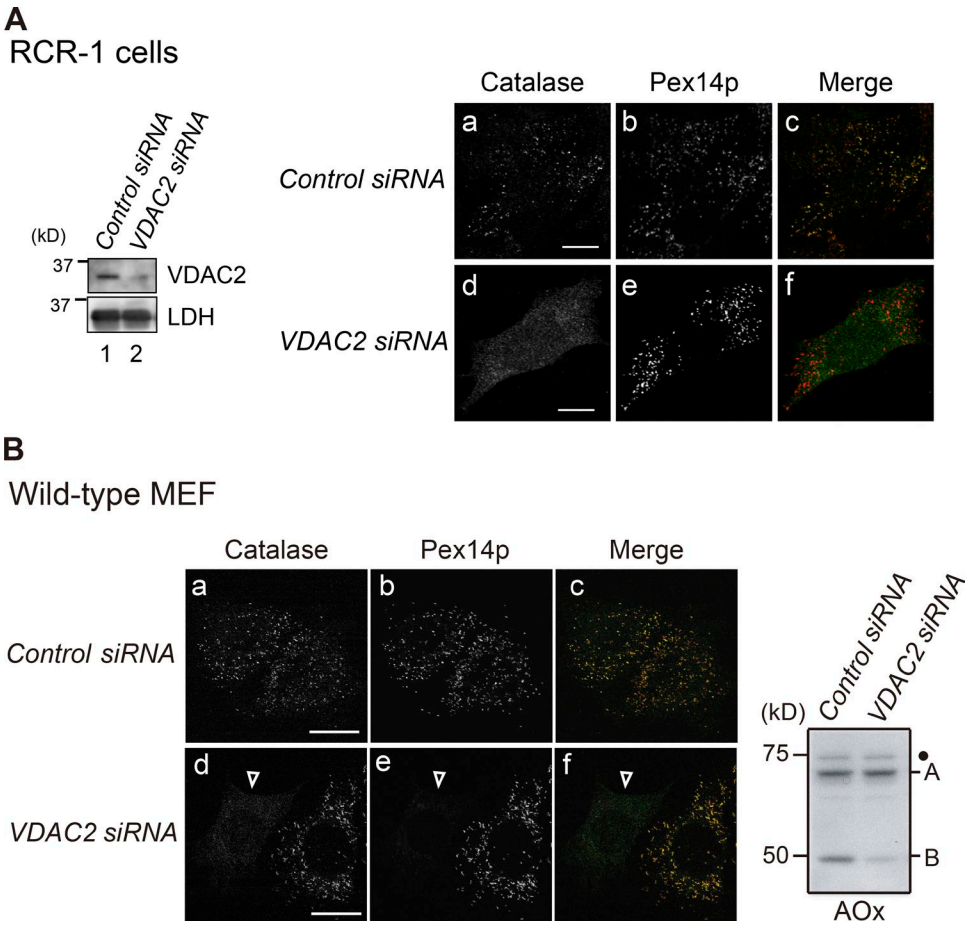

Figure S1. ***Vdac2* knockdown induces the defects of peroxisomal biogenesis.** (A, left) rat astrocytoma RCR-1 cells transfected with control siRNA and *VDAC2 siRNA* were subjected to Western blotting with antibodies to VDAC2 and lactate dehydrogenase (LDH). (Right) RCR-1 cells transfected with control siRNA and *VDAC2 siRNA* were immunostained with antibodies to catalase and Pex14p. Merged views of panels a plus b and panels d plus e are shown in c and f, respectively. Bars, 10 µm. (B) In control siRNA–transfected MEFs, normal peroxisomal morphology was observed as verified by the immunostaining of catalase and Pex14p. In *VDAC2 siRNA*–transfected cells, two different, wild-type and peroxisome-deficient, types of cells were observed. In one type of cells lacking peroxisomes (marked by arrowheads), catalase was mislocalized to the cytosol (d), and Pex14p was not detectable (e; arrowhead), apparently consistent with the phenotype of *Vdac2*$^{-/-}$ MEFs. Bars, 20 µm. Biogenesis of AOx was also verified by immunoblotting with anti-AOx antibody (right). A and B denote A and B chains, respectively; the dot indicates a nonspecific band.

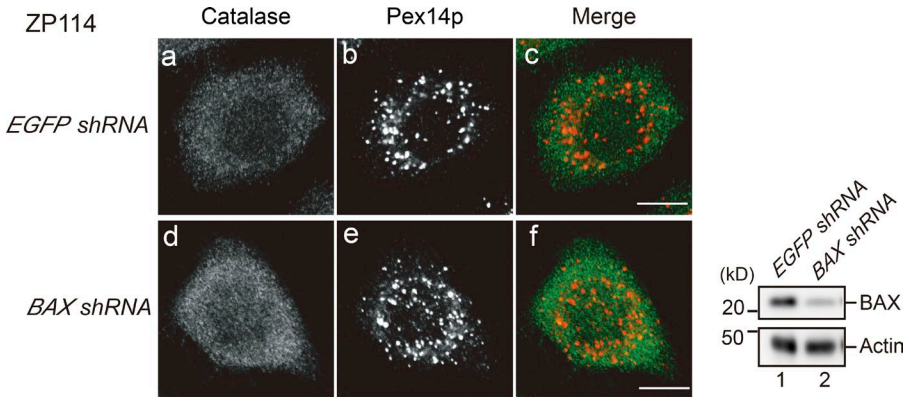

Figure S2. ***BAX* knockdown does not restore the impaired localization of peroxisomal matrix proteins in ZP114 cells.** (Left) ZP114 cells transfected with *EGFP shRNA* and *BAX shRNA* were immunostained with anticatalase and anti-Pex14p antibodies. Merged views of panels a plus b and panels d plus e are shown in c and f, respectively. (Right) ZP114 cells transfected with *EGFP shRNA* or *BAX shRNA* were analyzed by Western blotting with antibodies to BAX and actin. Bars, 10 µm.

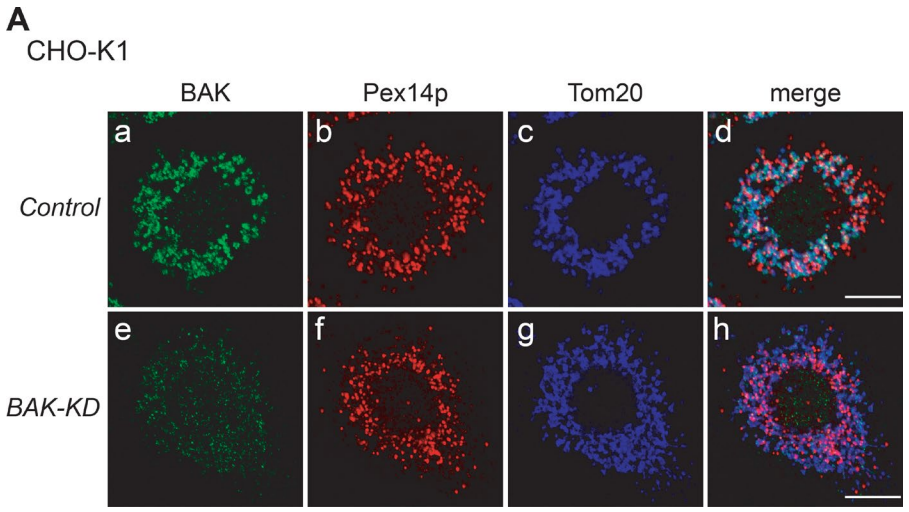

Figure S3. **BAK is mostly localized to mitochondria in wild-type CHO-K1 cells.** CHO-K1 cells stably expressing empty vector (control) and *BAK shRNA* vector (*BAK-KD*) were fixed and immunostained with antibodies to BAK, Pex14p, and Tom20. Merged views of a–c and e–g are shown in d and h, respectively. Bars, 10 µm.

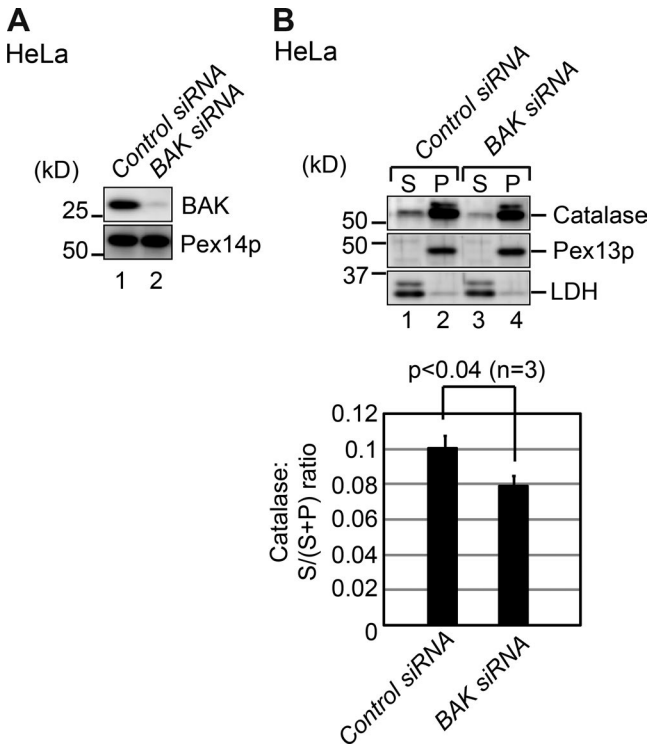

Figure S4. ***BAK* knockdown decreases cytosolic catalase in HeLa cells.** (A) HeLa cells transfected with control siRNA and *BAK siRNA* were analyzed by Western blotting with antibodies to BAK and Pex14p. (B, top) HeLa cells transfected with control siRNA and *BAK siRNA* were treated with digitonin to selectively permeabilize the plasma membrane. Permeabilized cells were centrifuged to separate into cytosol (S) and organelle (P) fractions. Equal aliquots of cytosol and organelle fractions were analyzed by Western blotting with antibodies to catalase, Pex13p, and lactate dehydrogenase (LDH). (Bottom) Catalase in cytosol and organelle fractions from control siRNA– and *BAK siRNA*–treated cells was quantified and shown as a ratio of cytosol/total (cytosol plus organelle). Data represent means ± SD.