Mukhopadhyay et al., http://www.jcb.org/cgi/content/full/jcb.200607061/DC1

Supplemental methods for Figure S1

Immunofluorescent microscopy

Cells were plated onto poly-L-lysine–coated coverslips in 60-mm dishes and transfected the next day with the indicated constructs using Effectene. 48 h later, the cells were fixed for 30 min at RT in a 4% PFA-buffered solution and permeabilized with 0.5% Triton X-100 for 10 min at RT. Staining with either the polyclonal PRDX3 C-terminal antibody and/or monoclonal HA antibody at a 1:300 dilution was performed for 1 h at RT in TBS-T containing 5% nonfat dairy milk. The species-specific fluorescein (FITC)- or Texas red–conjugated secondary antibodies (Abcam) at a 1:400 dilution were applied for 1 h at RT followed by counterstaining with DAPI and mounting together with Vectashield mounting medium (Vector Laboratories) for 10–15 min at RT in the dark. The cells were analyzed on a microscope (Axiovert 200M; Carl Zeiss MicroImaging, Inc.) equipped with digital CCD camera (C4742-95-12ERG; Hamamatsu) and the Openlab 3.1.5 and Volocity 2.1d19 software packages (Improvision) using a plan-Apochromat 63× NA 1.40 oil objective (Carl Zeiss MicroImaging, Inc.).

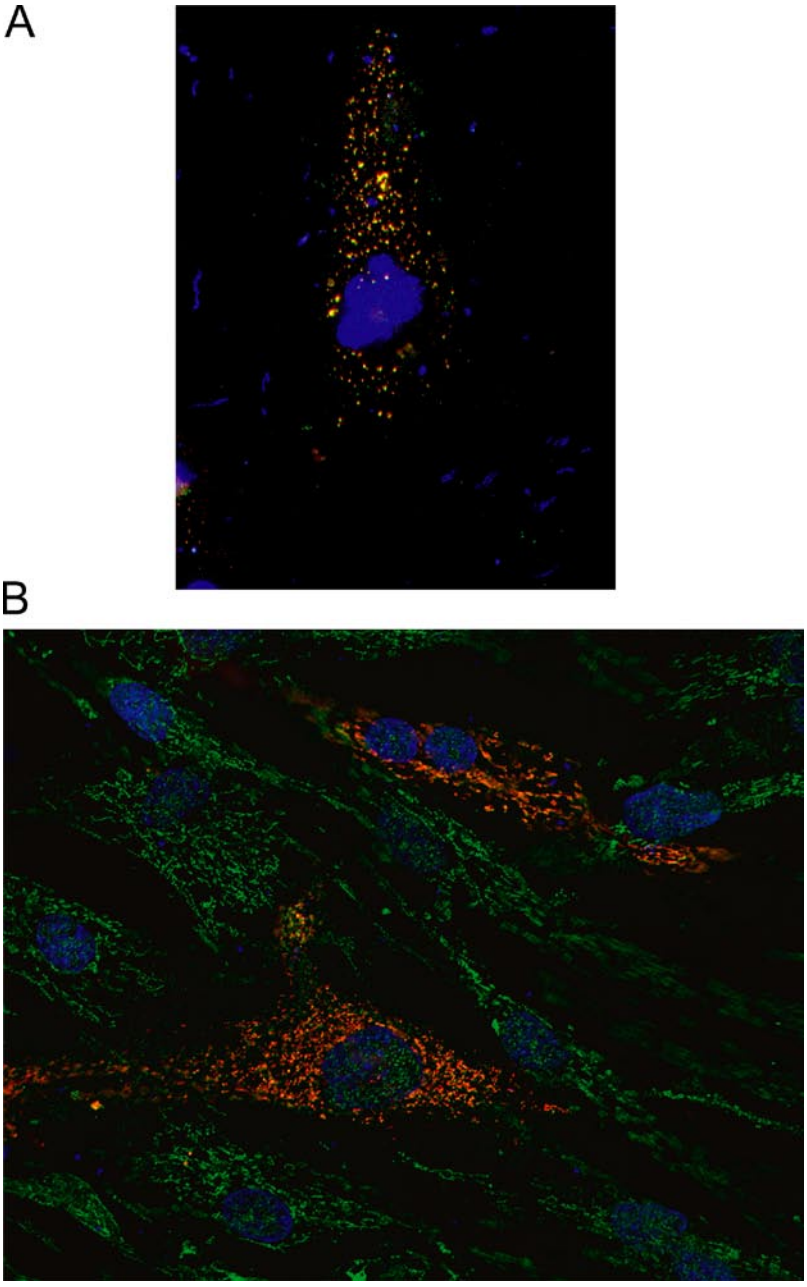

Figure S1. **Mislocalization of PRDX3 in FA-G mutant fibroblasts.** (A and B) Normal primary human fibroblasts (A) and FA-G mutant primary fibroblasts (PD352; B) were transfected with mitochondrial marker (pDsRed-Mito). The C-terminal PRDX3 antibody and FITC-labeled secondary antibody were used to detect the endogenous PRDX3 protein. Merge of the green and red images using a 40× objective.