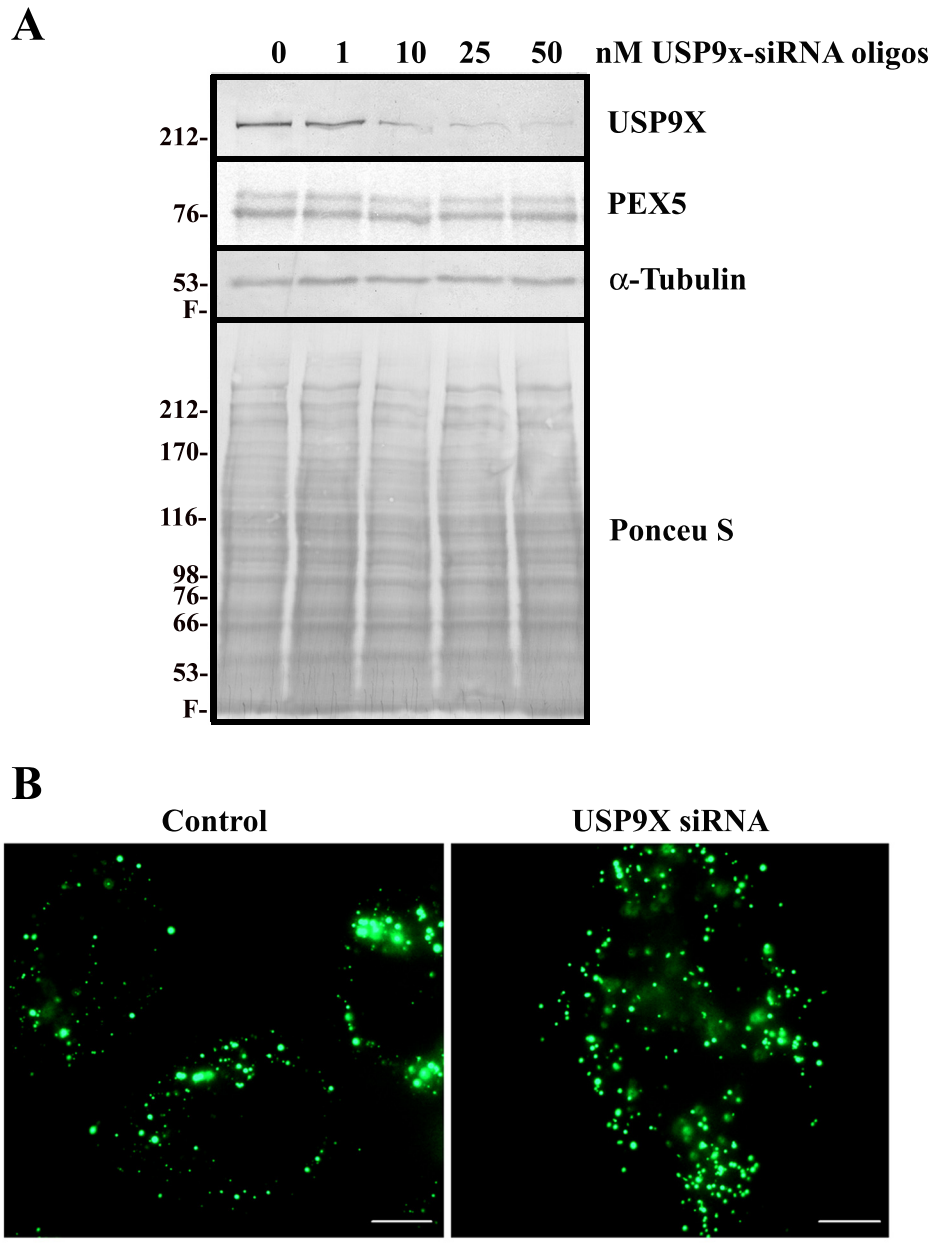


**FIGURE S3. USP9X knockdown in HeLa cells.** *A)* HeLa cells were transfected with different amounts of a 1:1 mixture of the two USP9X-specific siRNA oligos (see *Experimental Procedures* for details). The total concentrations of siRNA oligos used are indicated at the top. Three days after transfection, cells were harvested and analyzed by SDS-PAGE/Western blot. Appropriate sections of the Ponceau S-stained membrane (*lower panel*) were probed with antibodies directed to USP9X and α-tubulin, or subjected to blot overlay with $^{35}$S-labeled PEX14 to detect PEX5. The levels of USP9X in cells transfected with 25-50 nM of siRNA oligos were decreased by 90% as determined by densitometric analysis of Western blots. The knock down efficiency of USP9X was maximal at days 3 and 4 (data not shown). No significant decrease in PEX5 steady-state levels was observed in these knock down experiments. Note that the two PEX5 bands detected in HeLa cells probably correspond to the small and large isoforms of PEX5 (see Ref. 1 below). *B)* USP9X-knocked down HeLa cells were transfected with a plasmid encoding a peroxisomal matrix reporter protein (roGFP2-PTS1) 3 days after siRNA treatment and imaged 24 h later. Note the punctate distribution pattern of roGFP2-PTS1, characteristic of a peroxisomal localization. Control cells not subjected to the siRNA treatment are shown for comparison. *Bars* - 10 μm.

**REFERENCE**

1. Okumoto, K., Kametani, Y., and Fujiki, Y. (2011) Two proteases, trypsin domain-containing 1 (Tysnd1) and peroxisomal lon protease (PsLon), cooperatively regulate fatty acid beta-oxidation in peroxisomal matrix. *J. Biol. Chem.* **286**, 44367-44379