cancer resections with a marked decrease in the mean gray level value when compared to normal pancreas (B). All experiments using animals or human samples should be reviewed and approved by the Institutional animal care and use committee. Bar = 50 μm.

Figure 6. Sub-cellular localization of MnSOD by immunogold. MIA PaCa-2 human pancreatic cancer cells were infected with adenoviral vectors containing the cDNA for MnSOD. Ultrastructural examination was performed by Dr. Terry Oberley at the University of Wisconsin. Sections were treated with primary MnSOD antibody overnight, washed, and treated with gold-conjugated goat anti-rabbit immunoglobulin, fixed and stained. Labeling of MnSOD was extremely light in cells treated with the AdEmpty vector (A). MIA PaCa-2 cells treated with AdMnSOD demonstrated increases in labeling (arrow) in the mitochondria (bottom panel) (B). All experiments using animals or human samples should be reviewed and approved by the Institutional animal care and use committee. Bar = 5 μm

Figure 7. Glutathione peroxidase activity assay. The GPx assay is an indirect, coupled assay for glutathione peroxidase. This assay takes advantage of glutathione disulfide (GSSG) formed by the enzymatic action of GPx and is regenerated by excess glutathione reductase (GR) in the assay. The action of GR is monitored by following the disappearance of the co-substrate NADPH.

Supplementary method
Method for fixing tissues and cultured cells for immunogold immunohistochemistry

Fixing tissues for immunogold immunohistochemistry

1 Dice tissue into 1 mm cubes and place into glass scintillation vial. Immediately add 5 ml of Carson-Millonig’s fixative (4% (vol/vol) formaldehyde in 0.16 M sodium phosphate, pH 7.2) and fix for 1 h at room temperature. CAUTION All experiments using animals should be reviewed and approved by the Institutional animal care and use committee.

2 Remove the fixative and add 5 ml of Sorenson’s phosphate buffer (pH 7.4).

3 Embed the tissue in LR White resin (medium grade) and dehydrate through graded alcohols to 90% ethanol before immersing in a 2:1 mixture of LR White:90% ethanol and then undiluted LR White overnight at room temperature. Polymerize without accelerator at 56°C for 3 days.

4 Prepare ultrathin sections with an ultramicrotome.

5 Sections are then blocked with 4% BSA (wt/vol) and 0.5% (vol/vol) Tween 20 in Tris-buffered saline for 10 min.

6 Remove the blocking buffer and add primary antibody at a 1:200 dilution in antibody diluent and incubate the slides overnight at 4°C. Treat control sections with pre-immune serum, normal rabbit serum, or antibody diluent in place of the primary antiserum.

7 Wash the sections with PBS six times for five minutes at room temperature. Incubate the sections with gold-conjugated goat anti-rabbit IgG at a dilution of 1:50 for 90 min.

8 Wash the sections with PBS six times for five minutes at room temperature. Fix the sections with 2.5% (wt/vol) glutaraldehyde for 10 min before staining with 4% (wt/vol) uranyl acetate for 10 to 20 min. CAUTION glutaraldehyde toxic by inhalation and if swallowed, irritating to the eyes, respiratory system and skin. May cause sensitization by inhalation or skin contact. Wear gloves and lab coat in well ventilated area. CAUTION uranyl acetate is corrosive and irritating to eyes, skin, and mucous membranes. Wear nitrile gloves, eyewear, and lab coat.

9 Use a Hitachi 600 electron microscope to assess the ultrastructural labeling.

Fixing cultured cells for immunogold immunohistochemistry TIMING 4 d
1. Grow adherent cells in culture to 80-90% confluency.
2. Scrape the cells in 1 ml of PBS from the culture dish and pellet the cells in a 1.5 ml Microfuge tube. Centrifuge at 500 g for five minutes at room temperature.
3. Fix the cells by resuspending in 1 ml Carson-Millonig fixation at room temperature for 30 min to 2 h.
4. Pellet the cells at 500 g for five minutes at room temperature and decant fixative.
5. Rinse the cells in Sorensen’s phosphate buffer (0.2 M, pH 7.4) twice for 30 min at 4°C.
6. Embed the cells in paraffin using HistoGel following the manufacturer’s instructions.
7. Section the paraffin embedded blocks of cells as described in Box 6 (Steps 3-8).
